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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Antibiotic 107891, its factors a1 and a2, pharmaceutically acceptable salts and compositions, and use thereof

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ANTIBIOTIC 107891, ITS FACTORS A1 AND A2, PHARMACEUTICALLY ACCEPTABLE SALTS AND COMPOSITIONS, AND USE THEREOF.

The present invention concerns an antibiotic substance of microbial origin, arbitrarily denominated antibiotic 107891, which is a complex comprising -factors- A1 and A2, the pharmaceutical acceptable salts thereof, pharmaceutical compositions thereof and their use as an antibacterial agent.

Another object of the present invention is a process for preparing antibiotic 107891 which includes culturing *Microbispora* sp. 107891 (hereinafter identified as *Microbispora* sp. ATCC PTA-5024) or a variant or mutant thereof maintaining the ability to produce said antibiotic, recovering the antibiotic of the invention from the mycelium and/or from the fermentation broth, and isolating the pure substance by chromatographic means.

Antibiotic 107891 is a novel antimicrobial agent with a peptide structure containing lanthionine and methyllanthionine as constituents. These are the typical characteristics of lantibiotics and in particular of the subgroup acting primarily on cell wall biosynthesis.

Lantibiotics are peptides, which contain the thioether amino acid lanthionine as well as several other modified amino acids (H.G. Sahl and G.Bierbaum, (1998) "Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from gram-positive bacteria", Ann. Rev. Microbiol. 52:41-79). The majority of known lantibiotics have antibacterial activity, although some have been reported as active on different pharmacological targets. The antibacterial lantibiotics can be broadly divided into two groups on the basis of their structures: type-A lantibiotics are typically elongated, amphiphilic peptides, while type-B lantibiotics are compact and globular (O. McAuliffe, R.P. Ross and C. Hill, (2001): "Lantibiotics: structure, biosynthesis and mode of action",. FEMS Microb. Rev. 25: 285-308). Nisin is the typical representative of type A lantibiotic, whereas actagardine (gardimycin) and mersacidin belong to the type B lantibiotic

subclass. Both nisin-type and mersacidin-type lantibiotics interact with the membrane-bound peptidoglycan precursors lipid II, although the two classes differ in the effects they produce in the bacterial proliferation process. Nisin-type lantibiotics primarily kill bacteria by permeabilization of the cytoplasmic membrane (H. Brotz, M. Josten, I. Wiedemann, U. Schneider, F. Gotz, G. Bierbaum and H.G. Sahl, (1998):" Role of lipid-bound peptidoglycan precursors in the formation of pores by nisin, epidermin and other lantibiotics", Mol. Microbiol. 30:317-27), whereas mersacidin-type lantibiotics primary kill the bacterial cell by inhibiting the cell wall biosynthesis (H. Brotz, G. Bierbaum, K. Leopold, P.E. Reynolds and H.G. Sahl, (1998): "The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II", Antimicrob Agents Chemother. 42:154-60).

STRAIN AND FERMENTATION

Microbispora sp. 107891 was isolated in the environment and deposited on February 27, 2003 with the American Type Culture Collection (ATCC),10801 University Blvd, Manassas VA, 20110-2209 U.S.A., under the provision of the Budapest Treaty. The strain was accorded accession number PTA-5024.

The production of antibiotic 107891 is achieved by cultivating a *Microbispora* sp. strain capable of producing it, i.e. *Microbispora* sp. ATCC PTA-5024 or a variant or mutant thereof maintaining the ability to produce said antibiotic; isolating the resulting antibiotic from the culture broth and/or from the fermentation broth; and purifying the isolated antibiotic by chromatographic means. In any case, it is preferred to produce antibiotic 107891 under aerobic conditions in an aqueous nutrient medium containing easy assimilable sources of carbon, nitrogen, and inorganic salts. Many of the nutrient media usually employed in the fermentation field can be used, however certain media are preferred.

Preferred carbon sources are sucrose, fructose, glucose,

xylose, and the like. Preferred nitrogen sources are soybean meal, peptone, meat extract, yeast extract, tryptone, aminoacids, hydrolized casein and the like. Among the inorganic salts which can be incorporated in the culture media, there are the customary soluble salts capable of yielding sodium, potassium, iron, zinc, cobalt, magnesium, calcium, ammonium, chloride, carbonate, sulphate, phosphate, nitrate, and the like ions.

Preferably, the strain producing antibiotic 107891 is pre-cultured in a fermentation tube or in a shake flask, then the culture is used to inoculate jar fermentors for the production of substantial quantities of substances. The medium used for the pre-culture can be the same as that employed for larger fermentations, but other media can also be employed. The strain producing antibiotic 107891 can be grown at temperature between 17°C and 37°C, optimal temperatures being around 28-30°C.

During the fermentation, antibiotic 107891 production can be monitored by bioassay on susceptible microorganisms and/or by HPLC analyses. Maximum production of antibiotic 107891 generally occurs after circa 90 hours and before the 200 hours of fermentation.

Antibiotic 107891 is produced by cultivating *Microbispora* sp. ATCC PTA-5024 or a variant or mutant thereof capable of producing antibiotic 107891, and it is found in the culture broths and/or in the mycelium.

In this description and claims the term "antibiotic 107891", unless otherwise specified, identifies the antibiotic 107891 complex comprising factors A1 and A2.

MORPHOLOGICAL CHARACTERISTICS OF *Microbispora* sp. ATCC PTA-5024

Microbispora sp. ATCC PTA-5024 grows well on various standard solid media. Microscopic examination was measured using the culture grown on humic acid-Trace Salts Agar

(composition in g/l: humic acid 0.5, $FeSO_4*7H_2O$ 0.001, $MnCl_2*4H_2O$ 0.001, $ZnSO_4*7H_2O$ 0.001, $NiSO_4*6H_2O$ 0.001, MOPS 2, agar 20) added with 1 ml/l of vitamins solution (thiamine hydrochloride 25 mg/l, calcium pantotenate 250 mg/l, nicotinic acid 250 mg/l, biotin 0.5 mg/l, riboflavin 1.25 g/l, cyanocobalamin 6.25 mg/l, paraminobenzoic acid 25 mg/l, folic acid 500 mg/l, pyridoxal hydrochloride 500 mg/l).

In liquid culture (V6 medium, composition in g/l: dextrose 22, meat extract 5, yeast extract 5, casein 3, NaCl 1.5) no fragmentation of the mycelium is observed after 6 days of growth at 28°C. Microscopic examination on Humic acid-Trace Salts Agar (after 21 days of incubation at 28°C) reveals a branched, un-fragmented substrate mycelium and a monopodially branched aerial mycelium; many long, straight and poorly branched aerial hyphae are also visible. Characteristic longitudinal pairs of spores are borne by short sporophores laterally arising from branches or directly from the main aerial hyphae. Spores are globose and non-motile. Sporangium-like bodies or other particular structures are not observed.

CULTURAL CHARACTERISTICS OF *Microbispora* sp. ATCC PTA-5024

Microbispora sp. ATCC PTA-5024 was grown for six days in AF/MS liquid medium (see Example 1 for medium composition) at 28°C and 200 rpm, then transferred (5% inoculum) to a new AF/MS liquid medium and grown for further 6 days and finally inoculated (7% inoculum) into 100 ml of V6 liquid medium. After 6 days of growth at 28°C and 200 rpm, the mycelium was harvested by centrifugation and washed three times by sterile saline solution, then diluted to provide a suitable inoculum. Aliquots of the suspension were streaked in a cross-hatched manner onto various media recommended by Shirling and Gottlieb (E.B. Shirling and D. Gottlieb, (1966): "Method for Characterization of Streptomyces species", Int. J. Syst. Bacteriol. 16: 313-340), and media recommended by S.A. Waksman (1961): "The Actinomycetes", The Williams and Wilkins Co., Baltimore. Vol.2 :328-334).

The ability to use a variety of carbohydrates as a carbon

and energy source was determined using medium ISP4 without starch, added with 1 ml/l of the vitamin solution described above as basal medium; each carbon source was added at the final concentration of 1% (w/v).

NaCl tolerance, pH range of growth as well as ability to grow at different temperatures was determined onto ISP2 medium. All media were incubated at 28°C for three weeks; descriptions are referred to 21 days unless specified. Colour was assessed in natural daylight, using the Colour Atlas of Maerz and Paul (A. Maerz and M.R. Paul, 1950 - A Dictionary of Colour, 2nd edition. McGraw-Hill Book Co. Inc.,New York). Ability to reduce nitrates to nitrites was evaluated in sloppy Nitrate medium according to the procedure described by Williams *et al.* (S.T.Williams, M.Goodfellow, G.Alderson, E.M.H.Wellington, P.H.A.Sneath & M.J.Sackin, 1983 - Numerical classification of *Streptomyces* and related genera - J. Gen. Microbiol. 129, 1743-1813).

Growth, colonial appearance, substrate and aerial mycelium colour and pigment production for strain *Microbispora* sp. ATCC PTA-5024 are recorded in Table I. Vegetative growth is present on most of the media used, differently from the aerial mycelium that is present only on some of them. No evident pigmentation is shown on any medium used. Physiological characteristics of the strain are presented in Table II. Growth and aerial mycelium production are present at 17°C but not at 43°C. Production of aerial mycelium on ISP2 is present at pH higher than 6, while it is absent in presence of 1% NaCl.

The ability to use various carbohydrates for growth is shown in Table III.

Table I: growth characteristics of *Microbispora* sp. ATCC PTA-5024

MEDIUM	GROWTH & MORPHOLOGY	REVERSE COLOUR CODE
ISP 2 Yeast extract-Malt extract agar	Abundant growth, wrinkled surface; good production of pinkish (2A8) aerial mycelium. Slight production of orangish/light brown soluble pigment.	5 E 12 orangish/red
ISP 3 Oatmeal agar	Abundant growth; good production of pinkish (2A8) aerial mycelium, particularly on the arms of the cross-hatched streakes. Slight production of orangish soluble pigment.	11 H 10 orangish/pink
ISP 4 Inorganic salts-Starch agar	Good growth; no aerial mycelium produced. No soluble pigments produced. Starch hydrolysed.	11 I 9 orange
Glu/Asp Glucose-Asparagine agar	Discrete growth, thin; production of thin, beige/pinkish (9B4) aerial mycelium on the arms of the cross-hatched streakes. No soluble pigments produced.	12 K 12 orangish/light-brown
ISP 6 Peptone-yeast extract-iron agar	Scant growth, with pinkish single colonies grown in height, convolute, with a smooth surface; no aerial mycelium produced. No darkening of the medium.	nd

ISP 7 Tyrosine agar	Poor growth of a thin, orangish/light-brown substrate mycelium; no aerial mycelium produced. No darkening of the medium.	nd
ISP3+YE Oatmeal / 1% yeast extract agar	Abundant growth, wrinkled surface; very scant production of thin, pinkish aerial mycelium. No soluble pigments produced.	4 B 12 orangish/red

(ISP4 and Glucose-Asparagine agar added with 1 ml/L of vitamins solution)

Table II: physiological characteristics of *Microbispora* sp. ATCC PTA-5024.

TEST	**REACTION**
Starch hydrolysis	Positive
Casein hydrolysis	Negative
Calcium malate digestion	Negative
Litmus milk peptonization	Negative
Litmus milk coagulation	Negative
Gelatin liquefaction	Negative to slightly positive
Tyrosine reaction	Negative
Nitrate reduction	Positive
PH range of growth (14 days)	no growth at 4.2, good at 5.5 to 8.8; not tested out of this range. Aerial mycelium absent at pH ≤ 6.5

NaCl % tolerance	≤ 2; absence of aerial mycelium at ≥ 1.
Temperature range of growth	17°C to 37°C. Presence of aerial mycelium in the whole range; no growth at 43°C

Table III: utilization of carbon sources by *Microbispora* sp. ATCC PTA-5024.

Carbon source	**Growth (14 days)**
Arabinose	++
Cellulose	-
Fructose	++
Inositol	+/-
Mannitol	+++
Raffinose	-
Rhamnose	-
Sucrose	+++
Xylose	+++
Glucose	++
Glycerol	++
No sugar	-

+++ abundant; ++ good growth; + moderate growth; +/- scant growth; - no growth; aerial mycelium always absent.

CHEMOTAXONOMICAL CHARACTERISTICS OF *Microbispora* sp. ATCC PTA-5024

Microbispora sp. ATCC PTA-5024 *was* grown in GYM medium (glucose 4g/l; yeast extract 4 g/l; malt extract 10 g/l) at 28°C on a rotary shaker and the mycelium harvested, washed twice with sterile distilled water and subsequently freeze-dried. Analyses of amino acids were carried out according to the method of Staneck and Roberts, (J.L. Staneck and G.D. Roberts, (1974): "Simplified approach to identification of

aerobic actinomycetes by thin-layer chromatography", Appl. Microbiol. 28: 226-231). Menaquinones and polar lipids were extracted following the procedure of Minnikin et al. (D.E. Minnikin, A.G. O'Donnell, M. Goodfellow., G. Alderson, M. Athalye, A. Schaal and J.H. Parlett, (1984): "An integrated procedure of isoprenoid quinones and polar lipids", J. Microbiol. Meth.2: 233-241). Polar lipids were analysed by thin layer chromatography (D.E. Minnikin, V.Patel, L.Alshamaony, and M. Goodfellow, (1977): "Polar lipid composition in the classification of *Nocardia* and related bacteria", Int. J. Syst. Bacteriol. 27:104-117), menaquinones by HPLC (R.M. Kroppenstedt, (1982): "Separation of bacterial menaquinones by HPLC using reverse phase RP18 and a silver loaded ion exchanger as stationary phase", J. Liquid. Chromat. 5:2359-2367; R.M. Kroppenstedt, (1985): "Fatty acid and menaquinone analysis of actinomycetes and related organisms", in: Chemical Methods in Bacterial Systematics. No20 SAB Technical Series pp.173-199, M. Goodfellow and D.E. Minnikin eds, Academic Press, London) and fatty acid methyl esters by gas-liquid chromatography respectively (L.T. Miller, (1982): "A single derivatization method for bacterial fatty acid methyl esters including hydroxy acids", J. Clin. Microbiol.16: 584-586; M.Sasser, (1990): "Identification of bacteria by gas chromatography of cellular fatty acids", USFCC News Letters 20:1-6). The presence of mycolic acids was checked by the method of Minnikin et al.(D.E. Minnikin, L.Alshamaony, and M. Goodfellow, (1975): "Differentiation of *Mycobacterium, Nocardia* and related taxa by thin layer chromatographic analysis of whole organism methanolyzates",.J. Gen.Microbiol.88: 200-204).

Whole cell hydrolyzates of strain *Microbispora sp.* ATCC PTA-5024 contain *meso*-diaminopimelic acid as the diammino acid of the peptidoglycan. The predominant menaquinones are MK-9(III, VIII-H_4), MK-9(H_2)and MK-9(H_0).The polar lipid pattern

is characterized by the presence of phosphatidylethanolamine, methylphosphatidylethanolamine, phosphatidyl-glycerol, diphosphatidyl-glycerol, phosphatidyl-inositol, phosphatidyl-inositolmannosides and N-acetylglucosamine containing phospolipd ,i.e. phospholipid type IV according to Lechevalier et al.(H.A. Lechevalier, C. De Briève and M.P. Lechevalier, (1977): "Chemotaxonomy of aerobic actinomycetes: phospholipid composition", Biochem. Syst. Ecol. 5: 246-260). The major components of fatty acid pattern are *anteiso* 15:0, *iso* 16:0, *n*-16:0, *anteiso* 17:0, and 10-methyl-heptadecanoic (10-Me-17:0), i.e 3c *sensu* Kroppenstedt (R.M. Kroppenstedt, (1985): "Fatty acid and menaquinone analysis of actinomycetes and related organisms", in: Chemical Methods in Bacterial Systematics. No20 SAB Technical Series pp.173-199. M. Goodfellow and D.E. Minnikin eds, Academic Press, London). Mycolic acids are not detected.

IDENTITY OF STRAIN *MICROBISPORA* sp. ATCC PTA-5024.

The strain producing antibiotic 107891 is assigned to the genus *Microbispora,* family *Streptosporangiaceae* because of the following chemotaxonomical and morphological characteristics:

- presence of meso-diaminopimelic acid in the cell wall;
- major amount of MK-9(III, VIII-H_4) and phospholipid type IV according to Lechevalier et al.(H.A. Lechevalier, C. De Briève and M.P. Lechevalier, (1977): "Chemotaxonomy of aerobic actinomycetes: phospholipid composition", Biochem. Syst. Ecol. 5: 246-260);
- fatty acid profile of 3c *sensu* Kroppenstedt (R.M. Kroppenstedt, (1992): "The genus *Nocardiopsis*", in: The Prokariotes, Vol II, , pp.1139-1156, A. Balows, H. Truper, M. Dworkin, W. Harder and K.H. Schleifer eds; New York, Springer-Verlag);
- absence of mycolic acids;
- formation of characteristic longitudinal pairs of spores on the tips of short sporophores laterally branching from aerial hyphae. Non-motile spores.

As with other microorganisms, the characteristics of

strain producing antibiotic 107891 are subject to variation. For example, artificial variants and mutants of the strain can be obtained by treatment with various known mutagens, such as U.V. rays, and chemicals such as nitrous acid, N-methyl-N'-nitro-N-nitrosoguanidine, and many others. All natural and artificial variants and mutants of strain *Microbispora* sp. ATCC PTA-5024 capable of producing antibiotic 107891 are deemed equivalent to it for the purpose of this invention and therefore within the scope of invention.

EXTRACTION AND PURIFICATION OF ANTIBIOTIC 107891

As mentioned above, antibiotic 107891 is found almost equally distributed both in the mycelium and in the filtered fraction of the fermentation broth.

The harvested broth may be processed to separate the mycelium from the supernatant of the fermentation broth and the mycelium may be extracted with a water-miscible solvent to obtain a solution containing the 107891 antibiotic, after removal of the spent mycelium. This mycelium extract may then be processed separately or in pool with the supernatant according to the procedures reported hereafter for the supernatant fraction. When the water-miscible solvent may cause interferences with the operations for recovering the antibiotic from the mycelium extract, the water-miscible solvent may be removed by distillation or may be diluted with water to a non-interfering concentration.

The term "water-miscible solvent" as used in this application, is intended to have the meaning currently given in the art of this term and refers to solvents that, at the conditions of use, are miscible with water in a reasonably wide concentration range. Examples of water-miscible organic solvents that can be used in the extraction of the compounds of the invention are: lower alkanols, e.g. (C_1-C_3) alkanols such as methanol, ethanol, and propanol; phenyl (C_1-C_3) alkanols such as benzyl alcohol; lower ketones, e.g. (C_3-C_4)

ketones such as acetone and ethyl methyl ketone; cyclic ethers such as dioxane and tetrahydrofuran; glycols and their products of partial etherification such as ethylene glycol, propylene glycol, and ethylene glycol monomethyl ether, lower amides such as dimethylformamide and diethylformamide; acetic acid dimethylsulfoxide and acetonitrile.

The recovery of the compound from the supernatant of the fermentation broth of the producing microorganism is conducted according to known *per se* techniques which include extraction with solvents, precipitation by adding non-solvents or by changing the pH of the solution, by partition chromatography, reverse phase partition chromatography, ion exchange chromatography, molecular exclusion chromatography and the like or a combination of two or more of said techniques. A procedure for recovering the compounds of the invention from the filtered fermentation broth includes extraction of antibiotic 107891 with water-immiscible organic solvents, followed by precipitation from the concentrated extracts, possibly by adding a precipitating agent.

Also in this case, the term "water-immiscible solvent" as used in this application, is intended to have the meaning currently given in the art to said term and refers to solvents that, at the conditions of use, are slightly miscible or practically immiscible with water in a reasonably wide concentration range, suitable for the intended use.

Examples of water-immiscible organic solvents that can be used in the extraction of the compounds of the invention from the fermentation broth are:

alkanols of at least four carbon atoms which may be linear, branched or cyclic such as n-butanol, 1-pentanol, 2-pentanol, 3-pentanol, 1-hexanol, 2-hexanol, 3-hexanol, 3,3-dimethyl-1-butanol, 4-methyl-1-pentanol, 3-methyl-1-pentanol, 2,2-dimethyl-3-pentanol, 2,4-dimethyl-3-pentanol, 4,4-dimethyl-2-pentanol, 5-methyl-2-hexanol, 1-heptanol, 2-heptanol, 5-methyl-1-hexanol, 2-ethyl-1-hexanol, 2-methyl-3-hexanol, 1-octanol, 2-octanol, cyclopentanol, 2-cyclopentylethanol, 3-

cyclopenthyl-1-propanol, cyclohexanol, cycloheptanol, cyclooctanol, 2,3-dimethyl-cyclohexanol, 4-ethylcyclohexanol, cyclooctylmethanol, 6-methyl-5-hepten-2-ol, 1-nonanol, 2-nonanol, 1-decanol, 2-decanol, and 3-decanol; ketones of at least five carbon atoms such as methylisopropylketone, methylisobutylketone, methyl-n-amylketone, methylisoamylketone and mixtures thereof.

As known in the art, product extraction from the filtered fermentation broth may be improved by adjusting the pH at an appropriate value, and/or by adding a proper organic salt forming an ion pair with the antibiotic, which is soluble in the extraction solvent.

As known in the art, phase separation may be improved by salting the aqueous phase.

When, following an extraction, an organic phase is recovered containing a substantial amount of water, it may be convenient to azeotropically distill water from it. Generally, this requires adding a solvent capable of forming minimum azeotropic mixtures with water, followed by the addition of a precipitating agent to precipitate the desired product, if necessary. Representative examples of organic solvents capable of forming minimum azeotropic mixtures with water are: n-butanol, benzene, toluene, butyl ether, carbon tetrachloride, chloroform, cyclohexane, 2,5-dimethylfuran, hexane, and m-xylene; the preferred solvent being n-butanol.

Examples of precipitating agents are petroleum ether, lower alkyl ethers, such as ethyl ether, propyl ether, and butyl ether, and lower alkyl ketones such as acetone.

According to a preferred procedure for recovering antibiotic 107891, the filtered fermentation broth can be contacted with an adsorption matrix followed by elution with a polar, water-miscible solvent or a mixture thereof, concentration to an oily residue under reduced pressure, and precipitation with a precipitating agent of the type already mentioned above.

Examples of adsorption matrixes that can be conveniently

used in the recovery of the compounds of the invention, are polystyrene or mixed polystyrene-divinylbenzene resins (e.g. M112 or S112, Dow Chemical Co.; Amberlite® XAD2 or XAD4, Rohm & Haas; Diaion HP 20, Mitsubishi), acrylic resins (e.g. XAD7 or XAD8, Rohm & Haas), polyamides such as polycaprolactames, nylons and cross-linked polyvinylpyrrolidones (e.g. Polyamide-CC 6, Polyamide-SC 6, Polyamide-CC 6.6, Polyamide-CC 6AC and Polyamide-SC 6AC, Macherey-Nagel & Co., Germany; PA 400, M.Woelm AG, Germany); and the polyvinylpirrolidone resin PVP-CL, (Aldrich Chemie GmbH & Co., KG, Germany) and controlled pore cross-linked dextrans (e.g. Sephadex® LH-20, Pharmacia Fine Chemicals, AB). Preferably , polystyrene resins are employed, particularly preferred being the Diaion HP 20 resin.

In the case of polystyrene resins, polystyrene-divinylbenzene resins, polyamide resins or acrylic resins a preferred eluent is a water-miscible solvent or its aqueous mixtures. The aqueous mixtures can contain buffers at appropriate pH value.

Also in this case, the term "water-miscible solvent", as used in this description and claims , is intended to have the meaning currently given in the art to said term as described above.

The successive procedures for the isolation and purification of the antibiotic may be carried out on the pooled extracts from the broth supernatant and from the mycelium. For example, when the portion of the antibiotic product contained in the filtered fermentation broth or supernatant is recovered by absorption on an absorption resin and the portion of the antibiotic product contained in the mycelium is extracted therefrom with a water-miscible solvent, followed by adsorption onto an absorption resin, the eluted fractions from each of the two sets of absorption resins may be combined, optionally after concentration, and then further processed as a unitary crop. Alternatively, when the two sets of absorption resins utilized for the separate extraction stages are of the same type and have the same functional characteristics, they

may be pooled together and the mixture may be submitted to a unitary elution step, for instance, with a water-miscible solvent or a mixture thereof with water.

In any case, whatever may be the procedure adopted for recovering the crude antibiotic 107981, the successive purification step is usually carried out on the mixture of the crude materials resulting from the combination of the products originating from the separate extraction stages.

Purification of the crude 107891 antibiotic, can be accomplished by any of the known *per se* techniques but is preferably conducted by means of chromatographic procedures. Examples of these chromatographic procedures are those reported in relation to the recovery step and include also chromatography on stationary phases such as silica gel, alumina, activated magnesium silicate an the like or reverse phase chromathography on silanized silica gel having various functional derivatizations, and eluting with water miscible solvents or aqueous mixture of water-miscible solvents of the kind mentioned above.

For instance, preparative HPLC chromatography may be employed, using RP-8 or RP-18 as stationary phase and a mixture of $HCOONH_4$ buffer : CH_3CN as eluting system.
The active fractions recovered from the purification step are pooled together, concentrated under vacuum and precipitated by addition of a precipitating agent of the kind mentioned above.
As usual in this art, the production as well as the recovery and purification steps may be monitored by a variety of analytical procedures including inhibitory assay against susceptible microorganisms and analytical control using the HPLC or HPLC coupled with Mass spectrometry.

A preferred analytical HPLC technique is performed on a Waters instrument (Waters Chromathography, Milford, MA) equipped with a column Waters Simmetry-shield RP8, 5µ (250 x 4.6 mm) eluted at 1 ml/min flow rate and at 50°C temperature.

Elution was in isocratic mode with 30% phase B for 8 min, then with a linear gradient from 30 % to 60 % of phase B from 8 to 16 min and then up to 100% phase B from 16 to 20 min. Phase A was acetonitrile: 100 mM ammonium formate buffer (pH:5.0) 5:95 (v/v) and Phase B was acetonitrile.UV detector was at 282 nm.

The effluent from the column was splitted in a ratio 5:95 and the majority (ca. 950 µl/min) was diverted to photodiode array detector. The remaining 50 µl/min were diverted to the ESI interface of a Finnigan LCQ ion trap mass spectrometer (Thermoquest, Finnigan MAT, San Josè CA).

The mass spectrometric analysis was performed under the following conditions:

Sample inlet conditions:

Sheat gas (N_2) 60 psi;

Aux gas (N_2) 5 psi;

Capillary heater 250°C;

Sample inlet voltage settings:

Polarity both positive and negative;

Ion spray voltage +/- 5 kV;

Capillary voltage +/- 19V;

Scan conditions: Maximum ion time200 ms;

Ion time 5 ms;

Full micro scan 3;

Segment: duration 30 min, scan events positive (150-2000 m/z) and negative (150-2000 m/z).

In these analytical HPLC conditions the antibiotic 107891 A1 and A2 factors showed retention times of 13,2 min and 13,9 min, respectively. In the same HPLC system Ramoplanin A2 factor (L.Gastaldo, R.Ciabatti, F.Assi, E.Restelli, J.K.Kettenring, L.F.Zerilli, G.Romanò, M.Denaro and B.Cavalleri, (1992): "Isolation, structure determination and biological activity of A-16686 factors A'1, A'2 and A'3 glycolipodepsipeptide antibiotics", J. Ind. Microbiol. 11: 13-18) eluted with a retention time of 7.5 min.

Antibiotic 107891 factors A1 e A2 may be separated from a

purified sample of antibiotic 107891 complex by means of preparative HPLC.

Factor A1 was separated and purified on a Symmetry Prep. C18 column from the purified antibiotic 107891 complex dissolved in DMSO: formic acid 95:5 (v/v) using a 25 minutes linear gradient elution from 30% to 45% of phase B at 3.5 ml flow rate.

Phase B was acetobitrile. Phase A was 25 mM ammonium formate buffer pH 4.5: acetonitrile 95:5 (v/v). The eluted fractions containing pure antibiotic 107891 factor A1 were pooled and concentrated under vacuum. The residual solution was lyophilised yielding pure factor A1.

Factor A2 was separated and purified by isocratic elution on a Symmetry Prep. C18 column from a sample of purified antibiotic 107891 complex dissolved in acetic acid: acetonitrile: 100 mM ammonium formate buffer pH 4 50_120:80 (v/v) mixture. Isocratic elution was performed at a 7 ml flow rate with a mixture 100 mM ammonium formate buffer pH 4: acetonitrile in the proportion 82.5:17,5 (v/v). The eluted fractions containing pure antibiotic 107891 factor A2 were pooled and concentrated under vacuum. The residual solution was liophilized yielding pure factor A2.

Since antibiotic 107891 and its factors A1 and A2 contain basic functions, they are capable of forming salts with suitable acids according to conventional procedures and they may exist also in the free base form.

Antibiotic 107891 and its factors A1 and A2, when obtained in the free base form, may be converted with acids into the corresponding salts, which include non-toxic pharmaceutically acceptable salts. Suitable salts include those salts formed by standard reaction with both organic and inorganic acids such as, for example, hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, trifluoroacetic, trichloroacetic, succinic, citric, ascorbic, lactic, maleic, fumaric, palmitic, cholic, pamoic, mucic, glutamic, camphoric,

glutaric, glycolic, phthalic, tartaric, lauric, stearic, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic and the like acids. The addition salts of antibiotic 107891 and its factors A1 and A2, with acids can be prepared according to the usual procedures commonly employed. As an example, antibiotic 107891 or its factor A1 or its factor A2, in the free base form, is dissolved into the minimum amount of a suitable solvent, typically a lower alkanol, or a lower alkanol/water mixture, the stoichiometric amount of a suitable selected acid is gradually added to the obtained solution and the obtained salt is precipitated by the addition of a non-solvent. The addition salt which forms is then recovered by filtration or evaporation of the solvents.

Alternatively, these salts can be prepared in a substantially anhydrous form through lyophilization; in this case aqueous solutions containing the desired salts, resulting from the salification of antibiotic 107891 or its factor A1 or its factor A2 with a suitably selected acid in such a quantity as to obtain a pH comprised between 6.0 and 8.5 are filtered from any insolubles and lyophilized.

A specific addition salt may be also obtained from a solution of another salt form of antibiotic 107891 or its factor A1 or its factor A2 when the desired salt precipitates upon addition of the appropriate anion.

The transformation of the non salts compound of the invention into the corresponding addition salts, and the reverse, i.e. transformation of an addition salt of a compound of the invention into the non-salt form are within the ordinary technical skill and are encompassed by the present invention.

The formation of salts of antibiotic 107891 and its factors A1 and A2 may serve several purposes, including the separation, purification of said antibiotic 107891 and its factors A1 and A2 and their use as therapeutical agents or animal growth promoters. For therapeutical purposes, the pharmaceutically acceptable salts are usually employed.

The term "pharmaceutically acceptable salts" identifies those non-toxic salts which can be utilized in the therapy of warm-blooded animals.

The compounds of the invention can be administered as such or in mixtures with pharmaceutically acceptable carriers and can also be administered in conjunction with other antimicrobial agents such as penicillins, cephalosporins, aminoglycosides and glycopeptides.

Conjunctive therapy, thus includes sequential, simultaneous and separate administration of the active compound in a way that the therapeutic effects of the first administered one is not entirely disappeared when the subsequent is administered.

The compounds of the invention, or its pharmaceutically acceptable addition salts, can be formulated into forms suitable for parenteral, oral or topical administration. For i v. administration in the treatment of any infection involving a microorganism susceptible to the antibiotic, a parenteral formulation is, for instance, in water with an appropriate solubilising agent such as polypropylene glycol or dimethylacetamide and a surface-active agent (e.g. polyoxyethylene sorbitan mono-oleate or polyethoxylated castor oil) or cyclodextrins or phospholipid based formulations in sterile water for injection. An injectable formulation may be also obtained with an appropriate cyclodextrin.

The antibiotic may also be used in a suitable pharmaceutical form such as a capsule, a tablet or an aqueous suspension for oral administration or with conventional creams or jellies for topical applications. Besides their use as medicaments in human and veterinary therapy, the compounds of the invention can also be used as animal growth promoters. For this purpose, a compound of the invention is administered orally in a suitable feed. The exact concentration employed is that which is required to provide for the active agent in a

growth promotant effective amount when normal amounts of feed are consumed.

The addition of the active compound of the invention to animal feed is preferably accomplished by preparing an appropriate feed premix containing the active compound in an effective amount and incorporating the premix into the complete ration. Alternatively, an intermediate concentrate or feed supplement containing the active ingredient can be blended into the feed. The way in which such feed premixes and complete rations can be prepared and administered are described in reference books (such as "Applied Animal Nutrition", W.H. Freedman and CO., S. Francisco, U.S.A., 1969 or "Livestock Feeds and Feeding" 0 and B books, Corvallis, Ore., U.S.A., 1977).

PHYSICO-CHEMICAL CHARACTERISTICS OF ANTIBIOTIC 107891

A) Mass spectrometry

In MS experiments on a Thermofinnigan LCQ deca instrument fitted with an electrospray source, using Thermofinnigan calibration mix, antibiotic 107891 gives two doubly protonated ions at m/z=1124 and at m/z 1116 corresponding to lowest isotope composition of the complex factors A1 and A2, respectively. The electrospray conditions were: Spray Voltage: 4.7 kV; Capillary temperature: 220°C; Capillary Voltage: 3 V; Infusion mode 10 µl/min. Spectra were recorded from a 0.2 mg/ml solution in methanol/water 80/20 (v/v) with trifluoracetic acid 0,1% and are reported in Fig.1A (full scan low resolution spectrum) and 1B (zoom-scan high resolution spectrum).

B) Infrared spectrum of antibiotic 107891 recorded in KBr with a Bruker FT-IR spectophotometer model IFS 48, exhibits the following absorption maxima (cm^{-1}): 3263; 2929; 1661;1631; 1533; 1402; 1346; 1114; 1026; 676. Infrared spectrum is reported in Fig.2

C) The U.V. spectrum of antibiotic 107891, performed in

methanol/H_2O (in ratio 80:20) with a Perkin-Elmer spectrophotometer Lambda 16, exhibits two shoulders at 226 and 267 nm. UV spectrum is reported in Fig.3

D) ^{1}H-NMR spectrum was recorded in the mixture methanol-d4/H_2O 40/10 (pH 4.3 HCl) at 40°C on a Bruker AMX 600 spectrometer applying a water suppression sequence. As internal standard the residual signal of methanol-d4 at 3.31 ppm was considered.

The ^{1}H-NMR spectrum of antibiotic 107891 is reported in Fig.4.

^{1}H NMR spectrum of antibiotic 107891 dissolved in methanol - d4/H2O (0.01N HCl) 40/10 exhibits the following groups of signals (in ppm) at 600 MHz using MeOH-d4 as internal standard (3.31 ppm), [δ=ppm, multiplicity; (attribution)]: 0.93 d (CH_3), 0.98 d (CH_3), 1.07 t (overlapped CH_3's), 1.18 t (overlapped CH_3's), 1.26 s (CH_3), 1.30 t (overlapped CH_3's), 1.62-1.74 m (CH_2), 1.78 d (CH_3), 1.80 d (CH_3), 2.03 m (CH_2), 2.24 m (CH), 2.36 m (CH_2), 2.72 - 3.8 m (peptidic alpha CH's), 3.8 - 5.2 m (peptidic alpha CH's), 5.53 - 6.08 s (CH_2), 5.62 d (CH double bond), 6.42 m (CH), 6.92 d (CH double bond), 7.0 - 7.55 m (aromatic CH's), 7.62 - 10.4 d and m (aromatic and peptidic NH's).

E) ^{13}C-NMR spectrum was recorded in the mixture methanol-d4/H2O 40/10 (pH 4.3 HCl) at 40°C on a Bruker AMX 600 spectrometer using as internal standard the residual signal of methanol-d4 at 49.15 ppm. The ^{13}C-NMR spectrum bb decoupled of antibiotic 107891 is reported in Fig.5.

^{13}C NMR spectrum of antibiotic 107891 dissolved in methanol-d4/H2O (0.01N HCl) 40/10 exhibits the following groups of signals (in ppm) at 600 MHz using MeOH-d4 as internal standard (49.15 ppm), [δ=ppm; (attribution)]: 13.6 - 23.2 (aliphatic CH_3's), 26.16 - 73 (aliphatic CH_2's and peptidic alpha CH's), 105 - 136 (aromatic and double bonds CH's and quaternary carbons), 164.3- 176.3 (peptidic carbonyls).

AMINO ACIDS COMPOSITION OF ANTIBIOTIC 107891

Antibiotic 107891 was submitted to complete acidic hydrolysis (HCl 6N, 105°C, 24h) and amino acid components of the antibiotic resistant to acid treatment were identified. Acid labile amino acids are not detectable with this approach. The hydrolysate was studied by HPLC-MS and GC-MS analysis, after suitable derivatization, in comparison with a mixture of standard amino acids similarly derivatized. For HPLC analysis the hydrolyzed sample was treated with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ-Tag™ Fluor reagent kit), for GC anlysis with a mixture of 3N HCl in anhydrous methanol and trifluoroacetic anhydride.

The qualitative HPLC analysis was carried out on a liquid chromatography system with simultaneous DAD and MS detection. The HPLC method had the following conditions:

Column: AccQ-Tag™ (Waters C18 NovoPak 4µm 3.9 x 150mm)

Column temperature: 37°C

Flow: 1 mL/min.

Phase A: Ammonium acetate 140mM pH 5 (acetic acid)

Phase B: Water/acetonitrile 60/40 v/v

Elution program:

Time (min.)	0	5	30	35	40	41
%B	5	5	80	95	95	5

UV detection: 254nm

MS conditions were the following:

Spectrometer: Finnigan LCQ Deca equipped with standard electrospray source.

Capillary temperature: 250°C

Source voltage: 4.70 KV

Source current: 80 µA

Capillary voltage: -15V

The qualitative GC analysis was carried out on a gas cromatographer fitted with MS-EI detection.

The GC method had the following conditions:

Column: J & W Scientific DB-5, 30m x 0.254 mm ID x 0.25 µm FT

Carrier gas: helium

Injection mode: splitless

Injector temperature: 200°C

Transfer line temperature: 300°C

Temperature program: from 50°C to 100°C at 2.5°C/min (10 min), from 100°C to 250°C at 10°C/min (15 min), 15 min at 250°C

Injection volume: 1 µl

MS conditions were the following:

Spectrometr: Finnigan TSQ700

Ionisation mode: Electron impact

Voltage setting:

Filament current: 400 mA

Electron multiplier: 1400 V

Electron energy:70 eV

Positive ion mode

Scan condition:

Scan range:40-650 amu

Scan time:1 sec

In the LC/MS GC/MS chromatograms obtained on the hydrolysate of antibiotic 107891, the following amino acids "acid-resistant" were identified along with other unidentified peaks:

Lanthionine, methyllanthionine, glycine, proline, valine, aspartic acid (NMR studies indicate that this is a transformation product of asparagine ,which generates aspartic acid by hydrolysis) phenylalanine, leucine.

MASS SPECTROMETRY OF ANTIBIOTC 107891 FACTOR A1 AND FACTOR A2

Antibiotic 107892 factor A1 gives a doubly protonated ion at m/z=1124 and factor A2 at m/z 1116 corresponding to the

lowest isotope composition in MS experiments on a Thermofinnigan LCQ deca instrument fitted with an electrospray source, using Thermofinnigan calibration mix. The electrospray conditions were: Spray Voltage: 4.7 kV; Capillary temperature: 250°C; Capillary Voltage: 8 V; Infusion mode 10 µl/min. Spectra were recorded from a 0.1 mg/ml solution in acetonitrile/water 50/50 (v/v) with acetic acid 0,5% and are reported in Fig.6A (full scan low resolution spectrum) and 6B (zoom-scan high resolution spectrum) and in Fig 7A (full scan low resolution spectrum) and B (zoom-scan high resolution spectrum).

NMR SPECROSCOPY OF ANTIBIOTIC 107891 FACTOR A1 AND FACTOR A2

^{1}H-NMR spectra of Antibiotic 107892 factor A1 and factor A2 were recorded in the mixture $CD_3CN:D_2O$ (1:1) at 298 K on a Bruker AMX 600 spectrometer applying a water suppression sequence. As internal standard the residual signal of acetonitrile-d3 at 1.94 ppm was considered.

The ^{1}H-NMR spectrum of antibiotic 107891 factor A1 is reported in Fig.8.

^{1}H NMR spectrum of antibiotic 107891 factor A1, dissolved in $CD_3CN:D_2O$ (1:1), exhibits the following groups of signals (in ppm) at 600 MHz using CD_3CN as internal standard (1.94 ppm), [δ=ppm, multiplicity; (attribution)]: 0.84 d (CH_3), 0.89 d (CH_3), 0.94 t (overlapped CH_3's), 1.1 d (CH_3), 1.13 d (CH_3), 1.15 t (overlapped CH_3's), 149 m (CH_2), 1.69 d (CH_3), 1.75 m (CH_2), 2.11 m (CH), 2.26 m (CH), 2.5 m (CH_2), 2.68 - 3.8 m (peptidic CH_a's), 3.8 - 5.0 m (peptidic CH_a's), 5.45 - 6.17 s (CH_2), 5.58 d (CH double bond), 6.36 m (CH), 6.86 d (CH double bond), 7.0 - 7.45 m (aromatic CH's). The dimethyl sulfoxide signal is present at 2.58 ppm and the formate signal is also present at 8.33 ppm as impurities.

The ^{1}H NMR spectrum bb decoupled of antibiotic 107891 factor A2 is reported in Fig.9.

^{1}H NMR spectrum of antibiotic 107891 factor A2, dissolved in

CD_3CN/D_2O (1:1), exhibits the following groups of signals (in ppm) at 600 MHz using CD_3CN as internal standard (1.94 ppm), [δ=ppm, multiplicity; (attribution)]: 0.84 d (CH_3), 0.88 d (CH_3), 0.94 d (CH_3), 1.06 d (CH_3), 1.14 d (CH_3), 148 m (CH_2), 1.65-1.75 m (CH_2), 1.67 d (CH_3), 2.15 m (CH), 2.25 m (CH), 2.5 m (CH_2), 2.77 - 3.8 m (peptidic CH_α's), 3.8 - 4.9 m (peptidic CH_α's), 5.45 - 6.14 s (CH_2), 5.59 d (CH double bond), 6.34 m (CH), 6.84 d (CH double bond), 7.0 - 7.42 m (aromatic CH's). The dimethyl sulfoxide signal is present at 2.58 ppm and the formate signal is also present at 8.32 ppm as impurities.

^{13}C-NMR spectra of Antibiotic 107892 factor A1 and factor A2 were recorded in the mixture $CD_3CN:D_2O$ (1:1) at 298 K on a Bruker AMX 600 spectrometer using as internal standard the residual signal of acetonotrile-d3 at 1.39 ppm.

The ^{13}C-NMR spectrum of antibiotic 107891 factor A1 is shown in Fig.10. ^{13}C NMR spectrum of antibiotic 107891 factor A1, dissolved in $CD_3CN:D_2O$ (1:1), exhibits the following groups of signals (in ppm) at 600 MHz using CD_3CN as internal standard (1.39 ppm), [δ=ppm; (attribution)]: 13.6 - 23.03 (aliphatic CH_3's), 25.69 - 77.9 (aliphatic CH_2's and peptidic CH_α's), 105 - 137.3 (aromatic and double bonds CH's and quaternary carbons), 165.6- 176.6 (peptidic carbonyls).

The ^{13}C-NMR spectrum bb decoupled of antibiotic 107891 factor A2 is shown in Fig.11.

^{13}C-NMR spectrum of antibiotic 10891 factor A2, dissolved in $CD_3CN:D_2O$ (1:1), exhibits the following groups of signals (in ppm) at 600 MHz using CD_3CN as internal standard (1.39 ppm), [δ=ppm; (attribution)]: 13.6 - 22.9 (aliphatic CH_3's), 25.65 - 73 (aliphatic CH_2's and peptidic CH_α's), 105 - 137.3 (aromatic and double bonds CH's and quaternary carbons), 165.7- 176.1 (peptidic carbonyls).

IN VITRO BIOLOGICAL ACTIVITY OF ANTIBIOTIC 107891

Antimicrobial activity of the antibiotic 107891 was determined by the broth microdilution method according to the National Committee for Clinical Laboratory Standards recommendations (NCCLS, document M7-A5).

The strains used were clinical isolates or strains from American Type Culture Collection (ATCC). The result of the tests are reported in Table IV and Table V.

Antibiotic 107891 was dissolved in DMSO to obtain a 1000 µg/ml stock solution, and subsequently diluted in water to obtain working solution. The media used were cation-adjusted Mueller Hinton broth (CAMHB) for *Staphylococci, M. catarrhalis, Enterococci* and *L. monocytogenes;* Todd Hewitt broth (THB) for *Streptococci;* GC medium + 1% Isovitalex +1% haemine for *Neisseria spp.;* Brain Hearth Infusion +1% C supplement for *H. influenzae;* Lactobacillus broth for *Lactobacilli;* Middlebrook 7H9 with Middlebrook OADC enrichment for *M. smegmatis;* RPMI 1640 Medium for *C.albicans*. Wilkins Chalgren broth + oxyrase(1:25 v/v) for *Clostridia;* Brucella broth containing cisteine (0.5 g/L) for *Propionibacteria*. Inocula for bacteria were 10^5 CFU/ml. *C.albicans* inoculum was $1X10^4$ CFU/ml. All the tests were performed in presence of 0.02% of bovine serum albumin (BSA). Cultures were incubated at 35°C in air except *Clostridia* and *Propioniobacteria* strains that needed anaerobic atmosphere. After 18-24 hours visual readings were performed and MICs determined. The MIC was defined as the lower concentration of antibiotic at which there is no visible growth.

TABLE IV: Antimicrobial activity of 107891

Microorganism		MIC (µg/ml)
		107891
819	*Staph. aureus* Smith ATCC19636	≤ 0.13
4061	*Staph. aureus* LIM1	≤ 0.13
3798	*Staph.aureus* clin. isolate VISA	2
1400	*Staph. aureus* clin. isolate Met-R	≤ 0.13
613	*Staph. aureus* clin. isolate Met-R	≤ 0.13
3797	*Staph. aureus* clin. isolate VISA Me	2
4064	*Staph. aureus* LIM2 GISA Met-R	0.5
1729	*Staph. haemolyticus* Met-R	8
1730	Met-S	2
147	*Staph. epidermidis* ATCC12228	≤ 0.13
1139		4
44	*Strept. pneumoniae* Pen-S	≤ 0.13
2868	Pen-I	≤ 0.13
49	*Strept. pyogenes*	≤ 0.13
559	*Ent. faecalis* Van-S	1
560	*Ent. faecalis* Van-A	0.5
A533	*Ent. faecalis* Van-A	1
568	*Ent. faecium* Van-S	2
569	*Ent. faecium* Van-A	1
B518	*Ent. faecium* Van-A	2
A6345	*Ent. faecium* Van-A Lnz-R	4
3754	*Mycobacterium smegmatis*	32
884	*Listeria garviae*	≤ 0.13
148	*Listeria delbrueckii* ATCC4797	4
1450	*Listeria monocytogenes*	0,125
833	*Haemophilus influenzae*	32
970	*Haemophilus influenzae* ATCC 19418	32
3924	*Moraxella catharralis*	1
76	*Moraxella catharralis* ATCC8176	0.25
1613	*Neisseria meningitidis* ATCC13090	0,5
997	*Neisseria gonorrhaee*	0,25
47	*Escherichia coli*	>128
145	*Candida albicans*	>128

TABLE V: Antimicrobial activity of antibiotic 107891 against anaerobes bacteria

Microorganism	MIC (µg/ml)
	Antibiotic 107891
ATCC 27520 *Propionibacterium limphophilum*	0,015
ATCC 25564 *Propionibacterium granulosum*	0,03
ATCC 14157 *Propionibacterium propionicus*	4
P9 *Propionibacterium acnes*	0,125
1329 *Propionibacterium acnes*	0,5
ATCC 25746 *Propionibacterium acnes*	0,015
ATCC 6919 *Propionibacterium acnes*	0,125
ATCC 6922 *Propionibacterium acnes*	≤ 0.0039
ATCC 1348 *Propionibacterium acnes*	0,25
4018 *Clostridium difficile*	≤ 0.125
4025 *Clostridium difficile*	≤ 0.125
4022 *Clostridium difficile*	≤ 0.125
4032 *Clostridium perfringens*	≤ 0.125
4043 *Clostridium butyricum*	≤ 0.125
4009 *Clostridium beijerinckii*	≤ 0.125
4052 *Clostridium septicum*	≤ 0.125
60601 *Peptostreptococcus anaerobius*	>128

Antibiotic 107891 shows a good antibacterial activity against Gram-positive bacteria.

The MIC range against *Staphylococcus* spp., including Methicillin Resistant (MRSA) and Glycopeptides Intermediate (GISA) resistant strains, is = 0.13-4 µg/ml and against recent clinical isolates of *Enterococcus* spp., including Vancomycin Resistant (VRE), is 0.5-4 µg/ml. Against *Streptococcus* spp. MICs are ≤ 0.13 µg/ml.

Antibiotic 107891 is also active against anaerobic Gram-positive strains; the MICs are ≤ 0.13 µg/ml against *Clostridia* and ≤0.004-4 µg/ml against *Propionibacteria*. Antimicrobial activities were showed against *L.monocytogenes* (MIC 0.125

µg/ml) and *Lactobacilli* strains (MICs range ≤0.13-4 µg/ml). Some Gram-negative bacteria are susceptible to antibiotic 107891; MICs are 1-0.25 µg/ml versus *M. catharralis*, 0.5-0.25 µg/ml against *Neisseria* spp. and 32 µg/ml against *H. influenzae*.

Antibiotic 107891 is not active against the *E. coli* and *C. albicans* strains tested.

In time-kill experiments antibiotic 107891 shows bactericidal activity against *S.aureus* GISA and *E.faecalis* VanA strain; at 24 hours the bactericidal concentration is the MIC value in Mueller Hinton broth.

S.aureus can cause life-threatening infections and MRSA is of particular clinical significance because it is resistant to all penicillins and cephalosporins and also to multiple other antibiotics; in addition it easily spreads from patient to patient causing outbreaks of infection with important implications for healthcare facilities (W. Witte, (1999): "Antibiotic resistance in Gram-positive bacteria: epidemiological aspects", Journal of Antimicrobial Chemotherapy 44:1-9). The Centers for Disease Control (CDC) National Nosocomial Infection Surveillance System (NNIS) reported that methicillin resistance among *S. aureus* in US hospitals increased from 2.4% in 1975 to 29% in 1991, with a higher degree of resistance in intensive care units (L. Archibald, L.Philips, D.Monnet, J.E.Jr Mc Gowan, F. Tenover, R.Gaynes, (1997): "Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit", Clinic Infect. Dis. 24: 211-5). Nosocomial staphylococcal infections are associated with considerable morbidity and mortality, prolonging the duration of stay and increasing hospitalization costs. The majority of MRSA strains are resistant to several of the most commonly used antimicrobial agents, including macrolides, aminoglycosides, and the β-lactams antibiotics in

current use, including the latest generation of cephalosporins.

Vancomycin resistant hospital-acquired pathogens responsible for infections (such as endocarditis, meningitis and septicemia) are posing an increasing therapeutic challenge (Y.Cetinkaya, P.Falk and C.G.Mayhall, (2000): "Vancomycin-resistant enterococci", Clin. Microbiol. Rev. 13: 686-707; L.B.Rice, (2001): "Emergence of vancomycin-resistant enterococci",. Emerg. Infec. Dis.7:183-7).

S. pneumoniae and *M. catarrhalis* are recognized important pathogens of humans. They are a common cause of respiratory tract infections, particularly otitis media in children and lower respiratory tract infections in the eldery. *M. catarrhalis* and *S. pneumoniae* have been recently accepted as the commonest pathogens of the respiratory tract (M.C. Enright and H. McKenzy, (1997): "*Moraxella (Branhamella) catarrhalis*. Clinical and molecular aspect of a rediscovered pathogen", J. Med. Microbiol. 46:360-71).

Clostridia are responsible of different diseases: gas gangrene and related wound infections, tetanus, botulism, antibiotic associated diarrhea (CDAD) and pseumembranous colitis. Most of these microorganisms produce exotoxins that play an important role in the pathogenesis of the diseases. *C.difficile* is the causative agent responsible for 25% of cases of CDAD and for virtually all cases of pseudomembranous colitis. Over the last years the occurrence of *C.difficile* coinfection has occurred in patients with vancomycin resistant enteroccoccal infection or colonization (J.G. Bartlett, (1992): "Antibiotic associated diarrhea", Clinic.Infect.Dis. 15: 573-581).

IN VITRO BIOLOGICAL ACTIVITY OF ANTIBIOTIC 107891 FACTORS A1 AND A2

Table VI reports the antimicrobial activities of the individual factors A1 and A2 of antibiotic 107891. MICs were

determined by microbroth dilution method as above described

TABLE VI: Antimicrobiological activity of antibiotic 107891 Factors A1 and A2

Microorganism		MIC (µg/ml)	
		Factor A1	Factor A2
819	*Staph.aureus* Met-S	≤0.03	≤0.03
1524	*Staph.aureus* Met-R	≤0.03	≤0.03
2235	*Staph.aureus* Met-R	0,06	0,06
3894	*Staph.epidermidis* Met-R	≤0.03	0,06
3881	*Staph.epidermidis* Met-R	0,06	≤0.03
602	*Staph.haemolyticus* Met-R	0,25	0,25
3919	*Strept. pneumoniae* Pen-R	≤0.0015	≤0.0015
3915	*Strept.pneumoniae* Pen-S	≤0.0015	≤0.0015
4323	*Ent.faecalis* VanA	≤0.03	≤0.03
J1	*Ent.faecalis* VanA	1	1
4341	*Ent.faecalis VanB*	0,5	0,5
4397	*Ent.faecalis VanB*	1	1
4341	*Ent.faecalis VanB*	2	2
6349	*Ent.faecium* Van A LNZ-R	2	2
4	*Ent.faecium* Van A	1	1
3	*Ent.faecium* Van A	0,5	0,5
D561	*Ent.faecium* Van A	2	2
A8	*Ent.faecium* Van A	0,5	0,5
4339	*Ent.faecium* VanD	0,25	0,25
4174	*Ent.gallinarum*	1	1
997	*Neisseria gonorrhaee*	0,5	0,25
1613	*Neisseria meningitidis*	0,25	0,25
1016	*Propionibacterium acnes*	≤0.03	0,06

IN VIVO BIOLOGICAL ACTIVITY OF ANTIBIOTIC 107891

Female ICR mice (Harlan Italia SpA - S. Pietro al Natisone, Italy) weighing 23-25 g were used in experiments of acute lethal infection in immnunocompetent or neutropenic mice. Neutropenia was induced by two intraperitoneal administrations of cyclophosphamide, 200 and 100 mg/kg, at

four days and one day, respectively, before the mice were infected.

Infection was induced by inoculating intraperitoneally in immunocompetent mice (8 animals/dose/treatment group) a bacterial suspension of either a clinical isolate of methicillin resistant staphylococcus (*Staph. aureus* SA3817) or a standard methicillin susceptible strain (*Staph. aureus* Smith ATCC19636), or by inoculating in neutropenic mice a clinical isolate of glycopeptide resistant enterococcus (*Ent. faecalis* A533). The bacterial challenges (ca 10^6 cells/mouse) were given suspended in 0.5 mL of 5% bacteriological mucin (Difco). Untreated animals died within 24-72 h after infection. Antibiotic treatment began within 10-15 min after challenge. Antibiotic 107891 was administered once intravenously or subcutaneously in different aqueous formulations. The 50% effective dose (ED_{50}) and 95% confidence limits were calculated by the Spearman-Kärber method (D.J. Finney, (1952): "The Spearman-Kärber method", in: Statistical methods in biological assay. pp. 524-530, Charles Griffin & Co., Ltd., London) from the percentage of animals surviving at day 7. Results are reported in the following table.

Antibiotic 107891 is not toxic up to the maximum tested dose of 200 mg/kg.

Table VII ED_{50}s of antibiotic 107891 in acute lethal infections in mice.

Formulation	Strain	Route	ED_{50} mg/kg	95% confidence limits
A	MSSA	iv	2.1	1.7 - 2.7
		sc	2.1	1.7 - 2.7
A	VanA	iv	3.2	2.7 - 3.9
		sc	11.1	9.2 - 13.5
B	MRSA	sc	4.2	3.5 - 5.1
C	VanA	iv	3.7	2.8 - 4.9
		sc	12.7	10.7 - 15.0

Formulations:

A: 10% (v/v) DMSO, 10% (w/v) ß hydroxy-propyl cyclodextrin (Sigma), 80% (v/v) of 5% (w/v) glucose in H_2O

B: 10% (v/v) DMSO, 40% (v/v) PEG 400 in 0.1 M aqueous CH_3COOH

C: 50% (v/v) PEG 400 in H_2O

Strains:

I. MSSA: *Staph. aureus* Smith 819 ATCC19636

II. MRSA: *Staph. aureus* 3817, clinical isolate

III. VanA: *Ent. faecalis* A533, clinical isolate, in neutropenic mice

BRIEF DESCRIPTION OF THE DRAWINGS

FIG.1A (full scan low resolution spectrum) and 1B (zoom-scan high resolution spectrum) represent mass spectra of antibiotic 107891 showing a doubly protonated ion at m/z 1124 and m/z

1116.

FIG.2 represents the I.R. absorption spectrum of antibiotic 107891 dispersed in KBr.

FIG.3 represents the UV spectrum of antibiotic 107891 dissolved in methanol/H_2O.

FIG.4 represents the ^{1}H-NMR spectrum recorded in the mixture methanol-d4/H_2O 40/10 (pH 4.3 HCl) at 40°C on a Bruker AMX 600 spectrometer applying a water suppression sequence.

FIG. 5 represents the^{13}C-NMR spectrum recorded in the mixture methanol-d4/H_2O 40/10 (pH 4.3 HCl) at 40°C on a Bruker AMX 600 spectrometer.

The following examples further illustrate the invention and have not to be interpreted as limiting it in any way.

FIG.6A (full scan low resolution spectrum) and 6B (zoom-scan high resolution spectrum) represent mass spectra of antibiotic 107891 Factor A1 showing a doubly protonated ions $[M+2H]^{2+}$ at m/z 1124.

FIG.7A (full scan low resolution spectrum) and 7B (zoom-scan high resolution spectrum) represent mass spectra of antibiotic 107891 Factor A1 showing a doubly protonated ions $[M+2H]^{2+}$ at m/z 1116.

FIG.8 represents the ^{1}H-NMR spectrum of antibiotic 107891 Factor A1 recorded in the mixture $CD_3CN:D_2O$ (1:1) at 298 K on a Bruker AMX 600 spectrometer applying a water suppression sequence.

FIG.9 represents the ^{1}H-NMR spectrum of antibiotic 107891 Factor A2 recorded in the mixture $CD_3CN:D_2O$ (1:1) at 298 K on a Bruker AMX 600 spectrometer applying a water suppression sequence.

FIG. 10 represents the^{13}C-NMR spectrum of antibiotic 107891 Factor A1 recorded in the mixture $CD_3CN:D_2O$ (1:1) at 298 K on a Bruker AMX 600 spectrometer.

FIG. 11 represents the^{13}C-NMR spectrum of antibiotic 107891 Factor A2 recorded in the mixture $CD_3CN:D_2O$ (1:1) at 298 K on a Bruker AMX 600 spectrometer.

EXAMPLES

Example 1: Fermentation method of *Microbispora* sp. ATCC PTA-5024

Microbispora sp. ATCC PTA-5024 strain was maintained on oatmeal agar slants for 2-3 weeks at 28°C. The microbial content of one slant was scraped with 5 ml sterile water and inoculated into 500 ml Erlenmeyer flasks containing 100 ml of seed medium (AF/MS) which is composed of (g/l): dextrose 20, yeast extract 2, soybean meal 8, NaCl 1 and calcium carbonate 4. Medium was prepared in distilled water and pH adjusted to 7.3 prior to sterilization at 121°C for 20 min. The inoculated flasks were grown at 28°C, on a rotatory shaker operating at 200 rpm. After 4-6 days, 5% of this culture was inoculated into a second series of flasks containing the same fermentation medium. After 72 hours of incubation, 200 ml were transferred into 4 l bioreactor containing 3 l of the same vegetative medium.

The fermentation was carried out at 30°C, with 700 rpm stirring and 0.5 vvm aeration. After 72 hours the culture (1.5 l) was transferred into a 20 l bioreactor containing 15 l of the same vegetative medium. The fermentation was carried out for 48 hours at 30°C, at 500 rpm stirring and at 0.5 vvm aeration and then was transferred to the production tank.

The production of antibiotic 107891 was performed in a 300 l fermenter containing 200 l of the production medium M8 composed of (g/l): starch 20, glucose 10, yeast extract 2, casein hydrolysed 4, meat extract 2 and calcium carbonate 3. The medium was prepared in deionized water and the pH adjusted to 7.2 before sterilization at 121°C for 25 min. After cooling the fermenter was inoculated with about 14 l (7%) of pre-culture. Fermenter was run at 29°C, at 180 rpm stirring and at 0.5 vvm aeration with a head pressure of 0.36 bar. The fermenter was harvested after 98 hours of fermentation.

The production of the antibiotic 107891 was monitored by HPLC as previously described, after extraction of the whole culture broth with the same volume of methanol. The extraction was performed at room temperature under stirring for one hour.

Example 2: Alternative Fermentation method of *Microbispora* sp. ATCC PTA-5024

Microbispora sp. ATCC PTA-5024 was inoculated in 500 ml Erlenmeyer flasks containing 100 ml of growing medium (G1) consisting of g/l: glucose 10, maltose 10, soybean oil 10, soybean meal 8, yeast extract 2 and calcium carbonate 4. The medium was prepared in deionised water and sterilized at 120°C x 20 min. without pH adjustment. The inoculated flasks were incubated for 120-168 hours at 28°C, under 200 rpm stirring till a good growth was observed. The flasks were then used to inoculate (3 %) a 4 l bioreactor containing 3 l of seed medium AF/MS, which is composed as described in Example 1. After 120 hours of fermentation at 30°C, 700 rpm stirring and 0.5 vvm aeration, 1.5 l of the culture was transferred to a 20 l bioreactor containing 15 l of the same vegetative medium. The fermentation was carried out for 96 hours at 30°C, 600 rpm stirring and 0.5 vvm aeration, and was then transferred to the production tank.

The antibiotic production was obtained in a 300 l fermenter containing 200 l of the productive medium (V6) consisting of (g/l): dextrose 20, yeast extract 5, meat extract 5, hydrolysed casein 3, peptone 5 and NaCl 1.5. The medium was prepared in deionised water at pH adjusted to 7.5 with NaOH, and was sterilized at 121°C for 20 min.

The fermenter was inoculated with 14 l of seed culture (7%) and the fermentation was carried out at 29°C, stirred at 180 rpm, aerated with 100 l of standard air per minute (0.5 vvm). The antibiotic 107891 production was monitored by HPLC as previously described. The fermentation was harvested after about 160 hours.

Example 3 Recovery of antibiotic 107891

The fermentation broth described in the Example 1 was filtered by tangential filtration system (0.1 µm pore size membrane, Koch Carbo-Cor, Koch Wilmington, USA) to obtain 170 l of supernatant and 30 l of concentrated mycelium. Antibiotic 107891 complex was found both in the filtrate (A) and in the mycelium (B).

(A) The filtered broth was stirred one night at room temperature in the presence of Diaion HP-20 polystyrenic resin (4 l). The resin was then recovered, washed with 10 l methanol:water 4:6 (v/v) and eluted batchwise initially with 10 l methanol:water 9:1 (v/v) and then with 10 l methanol:butanol:water: 9:1:1 (v/v). The pooled eluted fractions containing antibiotic 107891 were concentrated to small volume on a rotary evaporator and then were freeze-dried, yielding 32 g of raw material. This raw material was dissolved in n-butanol (1 l) and then extracted three times sequentially with 800 ml water. The organic layer was concentrated under reduced pressure to an oily residue, which was dissolved in methanol. Upon addition of petroleum ether, 5 g of crude antibiotic preparation was obtained by precipitation.

(B) After addition of 25 l of methanol, the retentate portion containing themycelium was stirred 1 hour and was filtered to obtain 45 l of mycelium extract. This solution was then diluted with water (20 l) and was stirred one night at room temperature with Diaion HP-20 polystyrenic resin (1 l). The resin was then recovered, washed with 2 l methanol:water 40:60 (v/v) and eluted batch-wise sequentially with 3 l methanol:water 85:15 (v/v) and then with 2 l methanol:water 90:10 (v/v). The eluted fractions were monitored for the presence of antibiotic 107891 by agar diffusion assay on *Staphylococcus aureus* and by analytical HPLC method as previously reported.

The eluted fractions containing antibiotic 107891 were

pooled, were concentrated under reduced pressure and were freeze dryed, yielding 8.1 grams of crude antibiotic 107891.

Example 4: Alternative recovery of antibiotic 107891

The harvested broth from the 200 l tank fermentation described in example 2 was brought to pH 6.8 and the broth was filtered by tangential filtration (0.1 µ pore size membrane, Koch Carbo-Cor). The permeate (180 l) was stirred batch-wise overnight at room temperature with 2 l of Diaion HP20 resin (Mitsubishi Chemical) and the resin was then collected.

Methanol (25 l) was added to the retentate portion in the tangential filtration equipment (about 20 l) containing the concentrated mycelium. This suspension was stirred for 1 hour and then was filtered with the microfiltration system to a residual retentate volume of about 20 l. Additional methanol (25 l) was then added and the above process was repeated sequentially for a total of 5 cycles. The pooled methanol extracts (about 125 l) were diluted with 160 l of demineralized water and were stirred batch-wise overnight at room temperature with 3 l of Diaion HP 20 resin.. The resin was then collected, and was pooled with the Diaion HP 20 resin used to extract the broth permeate according to the process above described. The pooled resin was washed into a chromatographic column with 20 l of water:methanol 6:4 (v/v). The antibiotic 107891 was eluted with 23 l of methanol : 50 mM ammonium formate buffer pH 3.5 : n-butanol 9:1:1 (v/v). This eluate was then concentrated under vacuum to a final volume of 3 l. The concentrated solution was then loaded at pH 4.5 on a column of 2.5 l of polyamide CC 6 0.1-0.3 mm (Macherey-Nagel) conditioned with water:methanol 7:3 (v/v). The column was washed with water:methanol 7:3 (v/v) and then with 25 mM ammonium formate buffer pH 3.5 : methanol 7:3 (v/v). The antibiotic was eluted with water:methanol 3:7 (v/v) and then with 1:9 (v/v) mixture. The elution was completed with 25 mM ammonium formate buffer pH 2.8: methanol in the ratio 1:9 (v/v). The eluates containing antibiotic 107891 were pooled and concentrated under vacuum to a final volume of 1 l. The pH

of the concentrated solution was brought from 4 to 5.7 with 7 M ammonium hydroxide and then the mixture was centrifuged to collect the precipitate. This solid was suspended in water and freeze-dried, yielding 6.96 g of antibiotic 107891 preparation.

Example 5: Purification of antibiotic 107891

Crude antibiotic 107891 (3,6 g), prepared as described in Example 3, was purified by medium pressure chromatography on 100 g of reverse phase C8 (EC) 40-70 µm particle size, 60A pore size, IST (International Sorbent Technology, Mid-Glamorgan, UK) by using a Bùchi B-680 Medium Pressure Chromatography System (Bùchi laboratoriums-technik AG, Flawil Switzerland) equipped with B-687 gradient former, B-684 fraction collector, B-685 glass column 70 X 460 mm. The resin was previously conditioned with a mixture of phase A: phase B 8:2 (v/v) and was then eluted at 25 ml/min with 60 min linear gradient from 20 % to 60 % of phase B in 60 min.

Phase A was acetonitrile: 20 mM ammonium formate buffer (pH 6.6) 10: 90 (v/v); and phase B was acetonitrile: 20 mM ammonium formate buffer (pH: 6.6) 90: 10 (v/v).

The fractions containing antibiotic 107891 were pooled, concentrated under vacuum and lyophilized twice from water, yielding 430 mg of purified antibiotic 107891.

Example 6: Purification of individual factors A1 and A2 of antibiotic 107891

Factors A1 and A2 were purified from the antibiotic 107891 complex by preparative HPLC on a Symmetry Prep C18 (7 ìm particle size) column 7.8x300 mm Waters (Mildfold USA).

A) Factor A1 was purified by a 25 minutes linear gradient elution from 30% to 45% of Phase B, at 3.5 ml flow rate. Phase A was 25 mM ammonium formate buffer pH 4.5 : acetonitrile 95:5 (v/v) and Phase B was acetonitrile. Purified antibiotic 107891 complex (15 mg) was dissolved in 350 ìl of DMSO:formic acid 95:5 (v/v) and was processed per

chromatographic run. The A1 and A2 factors were typically eluted in 11-13 minutes. The eluted fractions of 14 chromatographic runs, containing pure antibiotic 107891 factor A1, were pooled and were concentrated under vacuum. The residual solution was lyophilized from water three times sequentially, yielding 15 mg of pure factor A1.

B) Factor A2 was purified by isocratic elution at 7 ml flow rate with 100 mM ammonium formate buffer pH 4 : acetonitrile 82.5 : 17.5 (v/v). Purified antibiotic 107891 complex (5 mg) was dissolved in 250 µl of acetic acid : acetonitrile : 100 mM ammonium formate buffer pH 4 50:120:80 (v/v) mixture and was processed per chromatographic run. The A1 and A2 factors were typically eluted in 9 - 10 minutes. The eluted fractions of 20 chromatographic runs, containing pure antibiotic 107891 factor A2, were pooled and were concentrated under vacuum. The residual solution was lyophilized twice from water yielding 8 mg of pure factor A2.

CLAIMS

1. Antibiotic 107891 having the following characteristics:

(A) Mass spectrum recorded from a 0.2 mg/ml solution in methanol/water 80/20 (v/v) with trifluoracetic acid 0,1% (Fig. 1A and 1B) on a Thermofinnigan LCQ deca instrument fitted with an electrospray source, using Thermofinnigan calibration mix under the following electrospray conditions: spray voltage: 4.7 kV; capillary temperature: 220° C; capillary voltage: 3V; infusion mode 10 µl/min, showing two double protonated ions at m/z 1116 and m/z 1124, corresponding to the lowest isotope composition of factor A1 and A2, respectively.

(B) Infrared spectrum (Fig. 2) recorded in Kbr with a Bruker FT-IR spectophotometer model IFS 48, exhibiting the following absorption maxima (cm^{-1}): 3263; 2929; 1661; 1631; 1533; 1402; 1346; 1114; 1026; 676;

(C) U.V. spectrum (Fig. 3), performed in methanol/H_2O (in ratio 80:20) with a Perkin-Elmer spectrophotometer Lambda 16, exhibiting two shoulders at 226 and 267 nm;

(D) ^{1}H-NMR spectrum (Fig. 4) recorded at 600 MHz in the mixture methanol-d4/H_2O 40/10 (pH 4.3 HCl) at 40°C on a Bruker AMX 600 spectrometer applying a water suppression sequence using as internal standard the residual signal of methanol-d4 at 3.31 ppm, exhibiting the following signals [δ=ppm muliplicity; (attribution)]:0,93 d (CH_3), 0.98 d (CH_3), 1.07 t (overlapped CH_3's), 1.18 t (overlapped CH_3's), 1.26 s (CH_3), 1.30 t (overlapped CH_3's), 1.62-1.74 m (CH_2), 1.78 d (CH_3), 1.80 d (CH_3), 2.03 m (CH_2), 2.24 m (CH), 2.36 m (CH_2), 2.72-3.8 m (peptidic alpha CH's), 3.8-5.2 m (peptidic alpha CH's), 5.53-6.08 s (CH_2), 5.62 d (CH double bond), 6.42 m (CH), 6.92 d (CH double bond), 7.0-7.55 m (aromatic CH's), 7.62-10.4 d and m (aromatic and peptidic NH's).

(E) ^{13}C-NMR spectrum (Fig. 5) recorded in the mixture methanol-d4/H_2O 40/10 (pH 4.3 HCl) at 40°C on a Bruker AMX 600 spectrometer, using as internal standard the residual signal

of methanol-d4 at 49.15 ppm, exhibiting the following signals: [δ=ppm; (attribution)]: 13:6-23.2 (aliphatic CH_3's), 26.16-73 (aliphatic CH_2's and peptidic alpha CH's), 105-136 (aromatic and double bonds CH's and quartenary carbons), 164.3-176.3 (peptidic carbonyls).

(F) The acid hydrolysate (HCl 6N, 105°C, 24 h) showing the presence of the following acid resistant amino acids after derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, lanthionine, methyllanthionine, glycine, proline, valine, aspartic acid (hydrolysis product of asparagine), phenylalanine, leucine;

and its salts with acids.

2) Factor A1 of antibiotic 107891 of claim 1 showing:

A) a doubly protonated ion at m/z 1124 corresponding to the lowest isotope composition in mass spectrum recorded from a 0,1 mg/ml solution in acetonitrile/water 50/50 (v/v) with acetic acid 0,5% (Fig. 6A and 6B) on a Thermofinnigan LCQ deca instrument fitted with an electrospray source, using Thermofinnigan calibration mix under the following electrospray conditions: spray voltage: 4.7 kV; capillary temperature: 250° C; capillary voltage: 8V; infusion mode 10 µl/min;

B) when dissolved in $CD_3CN:D_2O$ (1:1), the ^1H NMR spectrum (Fig.8) which exhibits the following groups of signals (in ppm) at 600 MHz using CD_3CN as internal standard (1.94 ppm), [δ=ppm, multiplicity; (attribution)]: 0.84 d (CH_3), 0.89 d (CH_3), 0.94 t (overlapped CH_3's), 1.1 d (CH_3), 1.13 d (CH_3), 1.15 t (overlapped CH_3's), 149 m (CH_2), 1.69 d (CH_3), 1.75 m (CH_2), 2.11 m (CH), 2.26 m (CH), 2.5 m (CH_2), 2.68 - 3.8 m (peptidic CH_a's), 3.8 - 5.0 m (peptidic $CH_á$'s), 5.45 - 6.17 s (CH_2), 5.58 d (CH double bond), 6.36 m (CH), 6.86 d (CH double bond), 7.0 - 7.45 m aromatic CH's);

C) when dissolved in $CD_3CN:D_2O$ (1:1), the ^{13}C NMR spectrum (Fig.10) which exhibits the following groups of signals (in

ppm) at 600 MHz using CD_3CN as internal standard (1.39 ppm), [δ=ppm; (attribution)]: 13.6 - 23.03 (aliphatic CH_3's), 25.69 - 77.9 (aliphatic CH_2's and peptidic CH_α's), 105 - 137.3 (aromatic and double bonds CH's and quaternary carbons), 165.6- 176.6 (peptidic carbonyls).

3. Factor A2 of antibiotic 107891 of claim 1 showing:

A) a doubly protonated ion at m/z 1116 corresponding to the lowest isotope composition in mass spectrum recorded from a 0,1 mg/ml solution in acetonitrile/water 50/50 (v/v) with acetic acid 0,5% (Fig. 7A and 7B) on a Thermofinnigan LCQ deca instrument fitted with an electrospray source, using Thermofinnigan calibration mix under the following electrospray conditions: spray voltage: 4.7 kV; capillary temperature: 250° C; capillary voltage: 8V; infusion mode 10 µl/min;

B) when dissolved in $CD_3CN:D_2O$ (1:1), the ^1H NMR spectrum (Fig. 9) which exhibits the following groups of signals (in ppm) at 600 MHz using CD_3CN as internal standard (1.94 ppm), [δ=ppm, multiplicity; (attribution)]: 0.84 d (CH_3), 0.88 d (CH_3), 0.94 d (CH_3), 1.06 d (CH_3), 1.14 d (CH_3), 148 m (CH_2), 1.65-1.75 m (CH_2), 1.67 d (CH_3), 2.15 m (CH), 2.25 m (CH), 2.5 m (CH_2), 2.77 - 3.8 m (peptidic CH_α's), 3.8 - 4.9 m (peptidic CH_α's), 5.45 - 6.14 s (CH_2), 5.59 d (CH double bond), 6.34 m (CH), 6.84 d (CH double bond), 7.0 - 7.42 m (aromatic CH's);

C) when dissolved in $CD_3CN:D_2O$ (1:1), the ^{13}C NMR spectrum (Fig.11), which exhibits the following groups of signals (in ppm) at 600 MHz using CD_3CN as internal standard (1.39 ppm), [δ=ppm; (attribution)]: 13.6 - 22.9 (aliphatic CH_3's), 25.65 - 73 (aliphatic CH_2's and peptidic CH_α's), 105 - 137.3 (aromatic and double bonds CH's and quaternary carbons), 165.7- 176.1 (peptidic carbonyls).

4. A process for producing antibiotic 107891 and its factors A1 and A2 and the salts thereof with acids as defined in claim 1

which comprises :

- cultivating *Microbispora sp.* ATCC PTA5024 or a variant or mutant thereof maintaining the ability to produce said antibiotic, under aerobic conditions, in an aqueous nutrient medium containing an assimilable source of carbon, nitrogen and inorganic salts;
- isolating the resulting antibiotic from the mycelium and/or the filtered fermentation broth;
- purifying the isolated antibiotic 107891 and, optionally, separating factor A1 and factor A2 therefrom.

5. A process according to claim 4, wherein the strain *Microbispora sp.* ATCC PTA 5024 or the antibiotic 107891 producing a variant or mutant thereof are pre-cultured.

6. A process according to any of claims 4 and 5, wherein the isolation of the antibiotic 107891 is carried out by filtering the fermentation broth and the antibiotic is recovered from the filtered fermentation broth according to a technique selected from: extraction with a water-immiscible solvent, precipitation by adding a non-solvent or by changing the pH of the solution, absorption chromatography, partition chromatography, reverse phase partition chromatography, ion exchange chromatography, molecular exclusion chromatography, and a combination of two or more of said techniques.

7. A process according to any of claims 4 and 5, wherein the isolation of the antibiotic 107891 is carried out by separating the mycelium from the supernatant of the fermentation broth and the mycelium is extracted with a water-miscible solvent whereby, after the removal of the spent mycelium, a water-miscible solution containing the crude antibiotic is obtained, which can be processed either separately or in pool with the antibiotic 107891 isolated from the filtered fermentation broth according to claim 6 to recover the antibiotic 107891 according to a technique selected from: extraction with a solvent, precipitation by adding a non-solvent or by changing the pH of the solution, absorption chromatography, partition chromatography, reverse

phase partition chromatography, ion exchange chromatography and molecular exclusion chromatography, or a combination of two or more of said techniques.

8. A process as in claim 7 whereby the concentration of the water-miscible solvent in the mycelium extract is reduced before it is processed to recover the antibiotic therefrom.

9. A process according to claim 6 whereby the filtered fermentation broth is contacted with an absorption resin, preferably a polystyrene, a mixed polystyrene-divinylbenzene or a polyamide resin, and said resin is eluted with a polar, water-miscible solvent or a mixture thereof with water, whereby a solution containing the crude antibiotic 107981 is obtained.

10. A process as in any of claims 7 and 8 wherein the mycelium is extracted with a C_1-C_3 alkanol, preferably methanol, and the mycelium extract is contacted with an absorption resin, preferably a polystyrene resin, and eluted therefrom with a polar water-miscible solvent or a mixture thereof with water, whereby a solution containing the crude antibiotic 107891 is obtained.

11. A process as in any of claims 4 and 5, wherein the solutions containing the crude antibiotic 107891 obtained according to each of claims 9 and 10 are pooled and processed for further purification of said antibiotic 107891.

12. A process as in any of claims 9, 10 and 11, wherein the solution containing the crude antibiotic 107981 is concentrated and then freeze-dried to yield a crude antibiotic 107891 solid product.

13. A process as in any of claims 4 and 5, wherein the absorption resins containing the absorbed antibiotic according to each of claims 9 and 10, respectively, are pooled and their mixture is eluted with a polar, water-miscible solvent or a mixture thereof with water.

14. A process according to any of claims 4 to 13 wherein the

antibiotic 107981 is purified by means of a chromatographic procedure, preferably by preparative HPLC or medium pressure chromatography.

15. A process according to any claims 4 to 14, wherein factor A1 and factor A2 are separated by preparative HPLC from the purified antibiotic 107891.

16. A pharmaceutical composition comprising an antibiotic selected from antibiotic 107891, its factor A1 and its factor A2 according to any of claims 1 to 3 or a pharmaceutically acceptable salt thereof with an acid.

17. A pharmaceutical composition according to claim 16, comprising a pharmaceutically acceptable carrier.

18. The antibiotic 107891, its factor A1 or its factor A2, according to any of claims 1 to 3 or a pharmaceutically acceptable salt thereof with an acid for use as a medicament.

19. Use of antibiotic 107891, its factor A1 or its factor A2, according to any of claims 1 to 3 or a pharmaceutically acceptable salt thereof with an acid for the manufacture of a medicament for the treatment or prevention of bacterial infections.

20. Use of the antibiotic 107891, its factor A1 or its factor A2 according to any of claims 1 to 3 and a non-toxic salt thereof with an acid as animal growth promoter.

21. A biologically pure culture of the strain *Microbispora sp.* ATCC PTA-5024, or a variant or mutant thereof maintaining the ability to produce the antibiotic of claim 1 when cultivated under submerged aerobic conditions in the presence of assimilable sources of carbon, nitrogen and inorganic salts.

ABSTRACT

The invention relates to an antibiotic substance of microbial origin, arbitrarily denominated 107891 which is produced by fermentation of *Microbispora* sp. ATCC PTA-5024, the pharmaceutically acceptable salts and compositions thereof, and their use as an antibacterial agent having inhibitory activity versus susceptible microbes.

Antibiotic 107891, which is a complex comprising two factors, denominated factors A1 and A2, has a peptide structure containing lanthionine and methyllanthionine as constituents which are typical characteristics of the antibiotics of the lantibiotics group.

Antibiotic 107891 shows a good antibacterial activity against Gram-positive bacteria including methicillin resistant and vancomycin resistant strains, and is active also against some Gram-negative bacteria such as *M. catharralis*, *Neisseria* species and H. *influenzae* and Mycobacteria.

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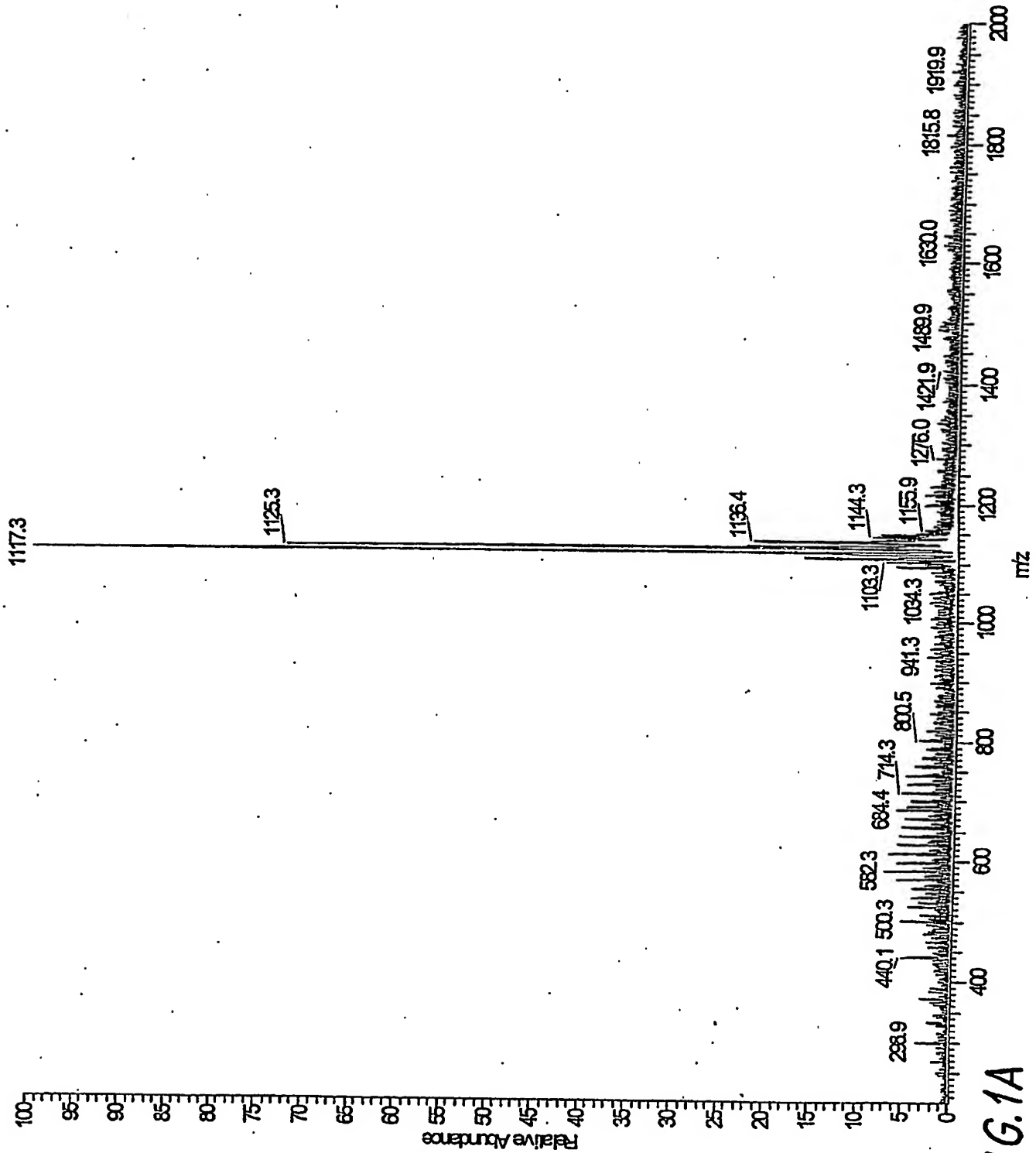
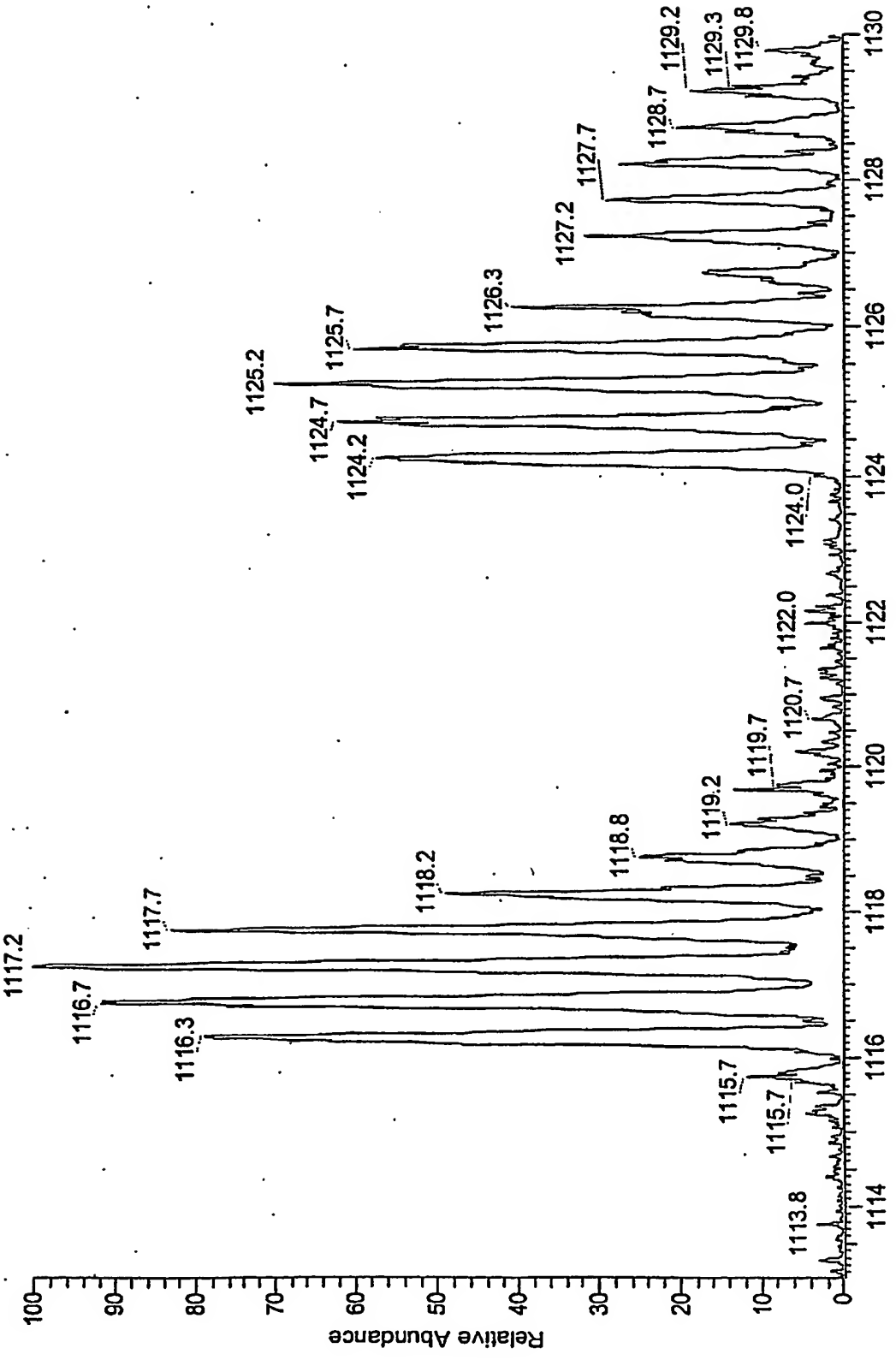


FIG.1A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20
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1124
1126
1128
1130
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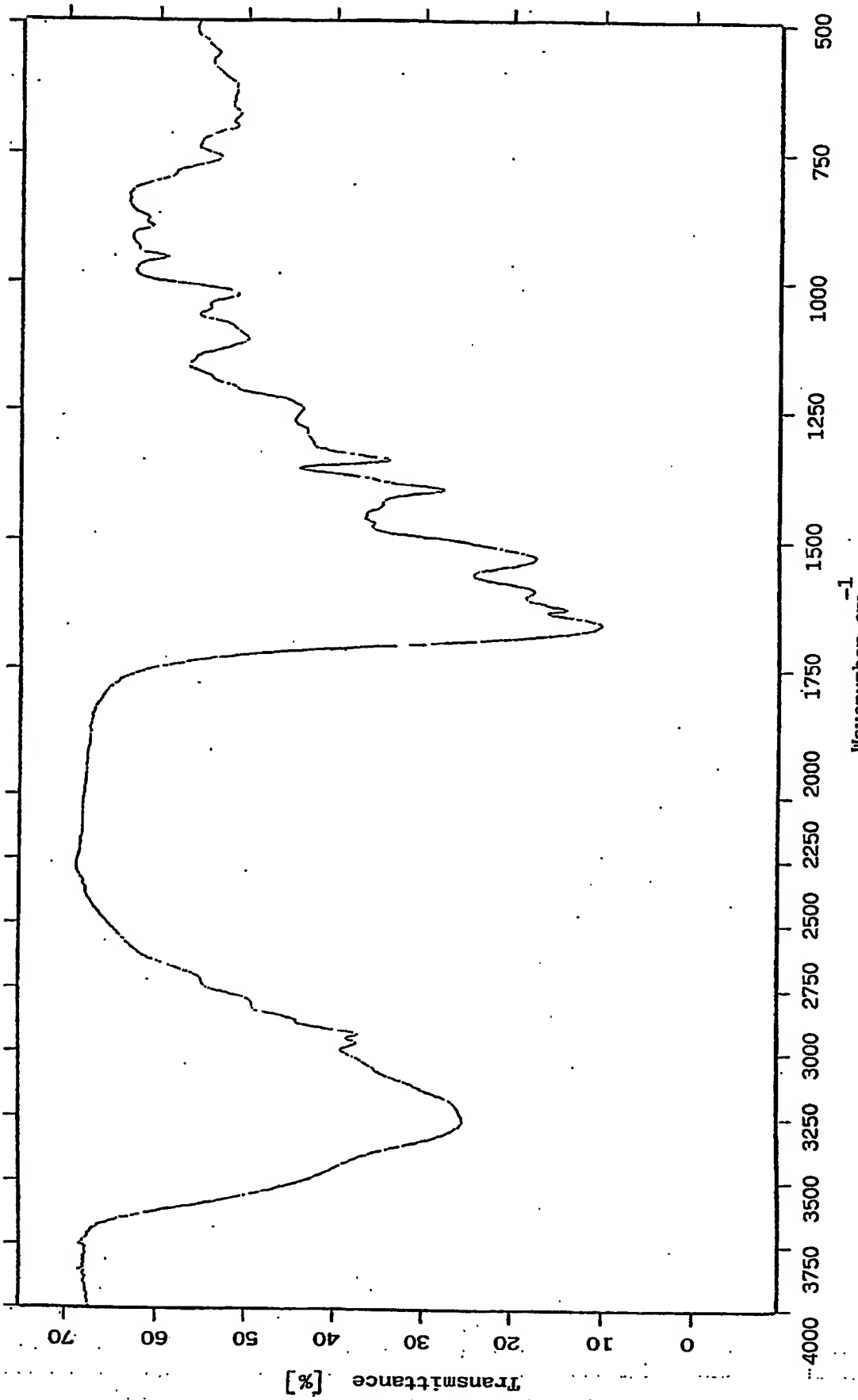
FIG. 1B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30
40
50
60
70
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3750
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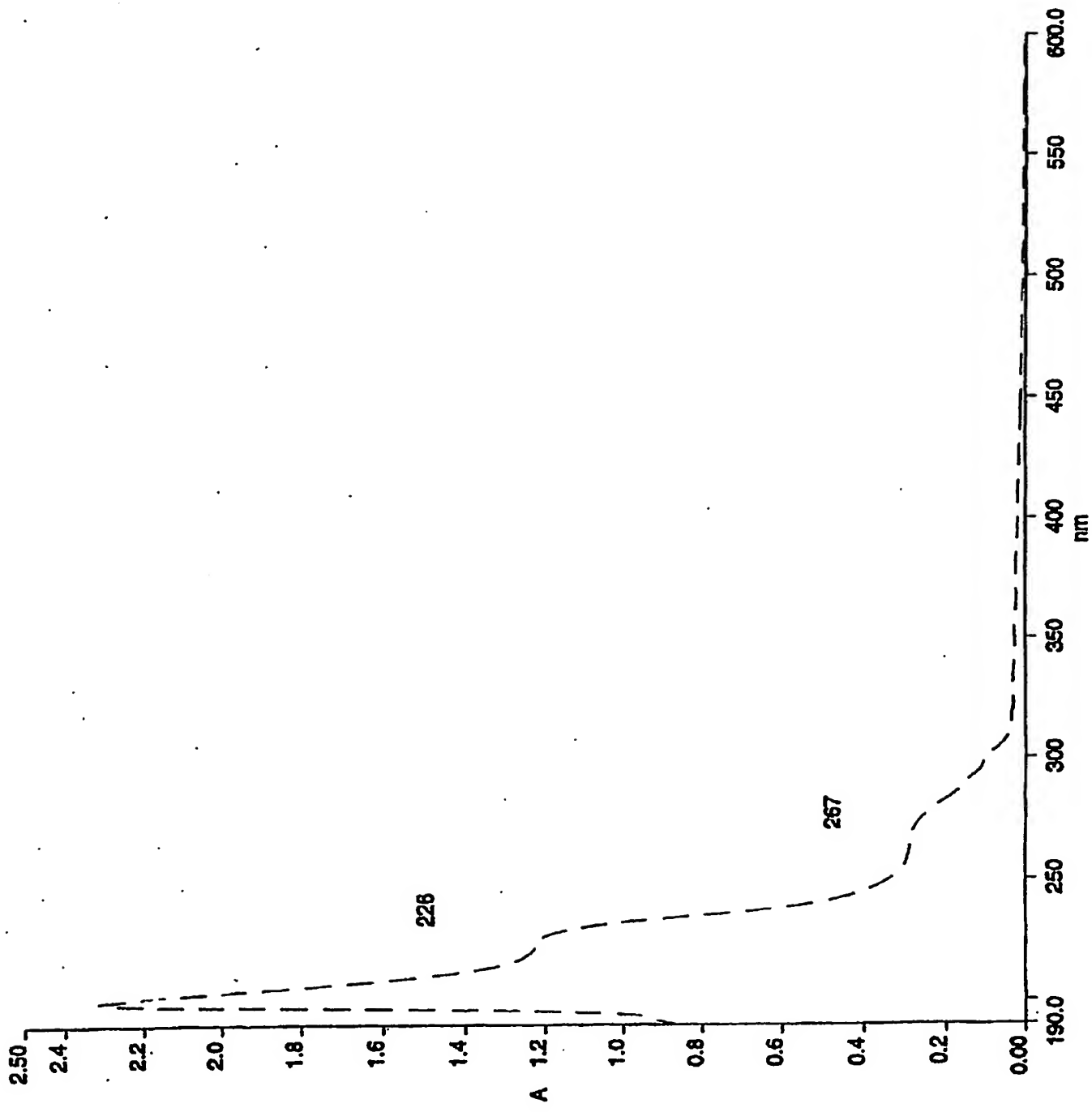
1500
1250
1000
750
500
Wavenumber cm^{-1}

FIG. 2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300
350
400
nm
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500
550
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226
267

FIG. 3

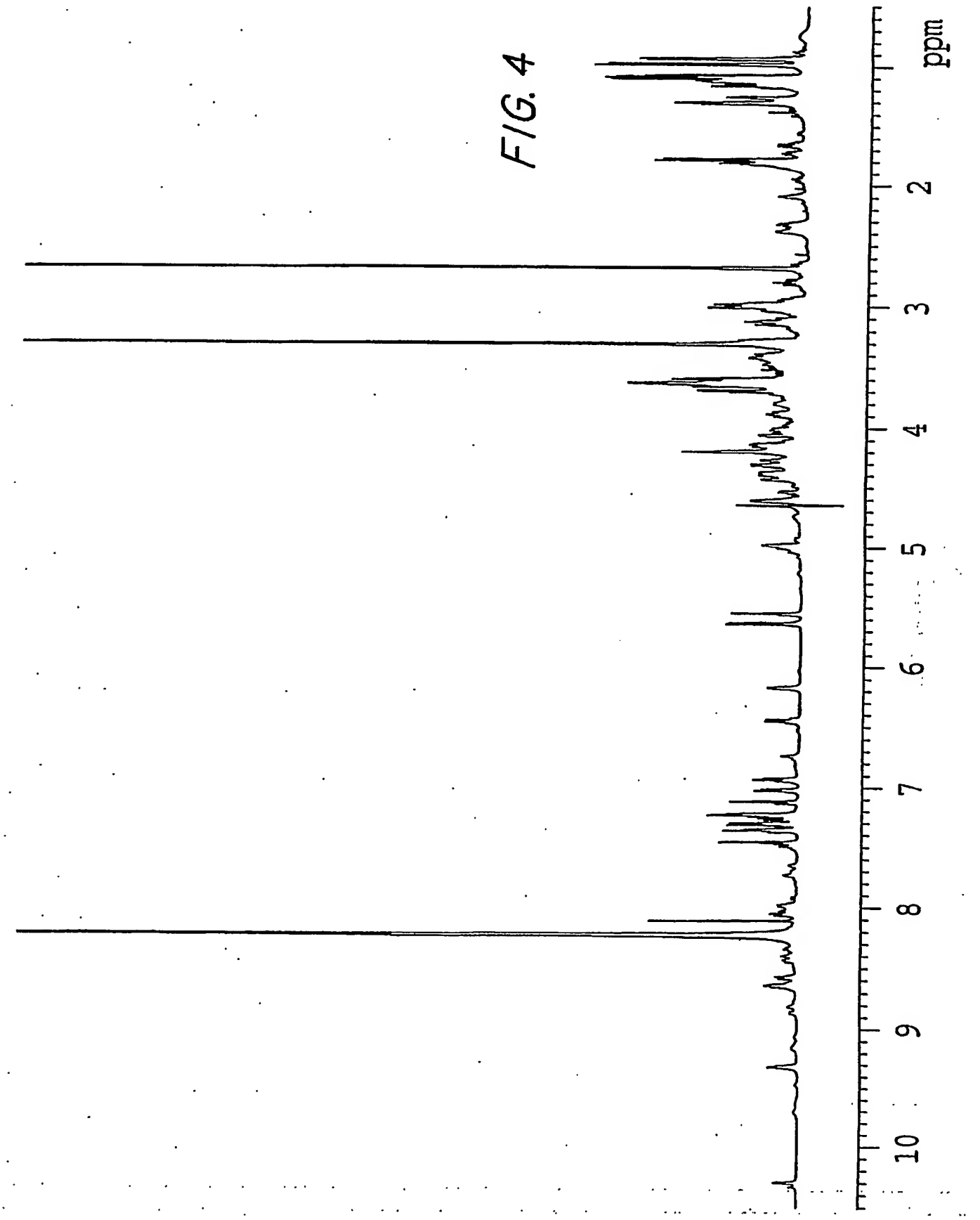


FIG. 4

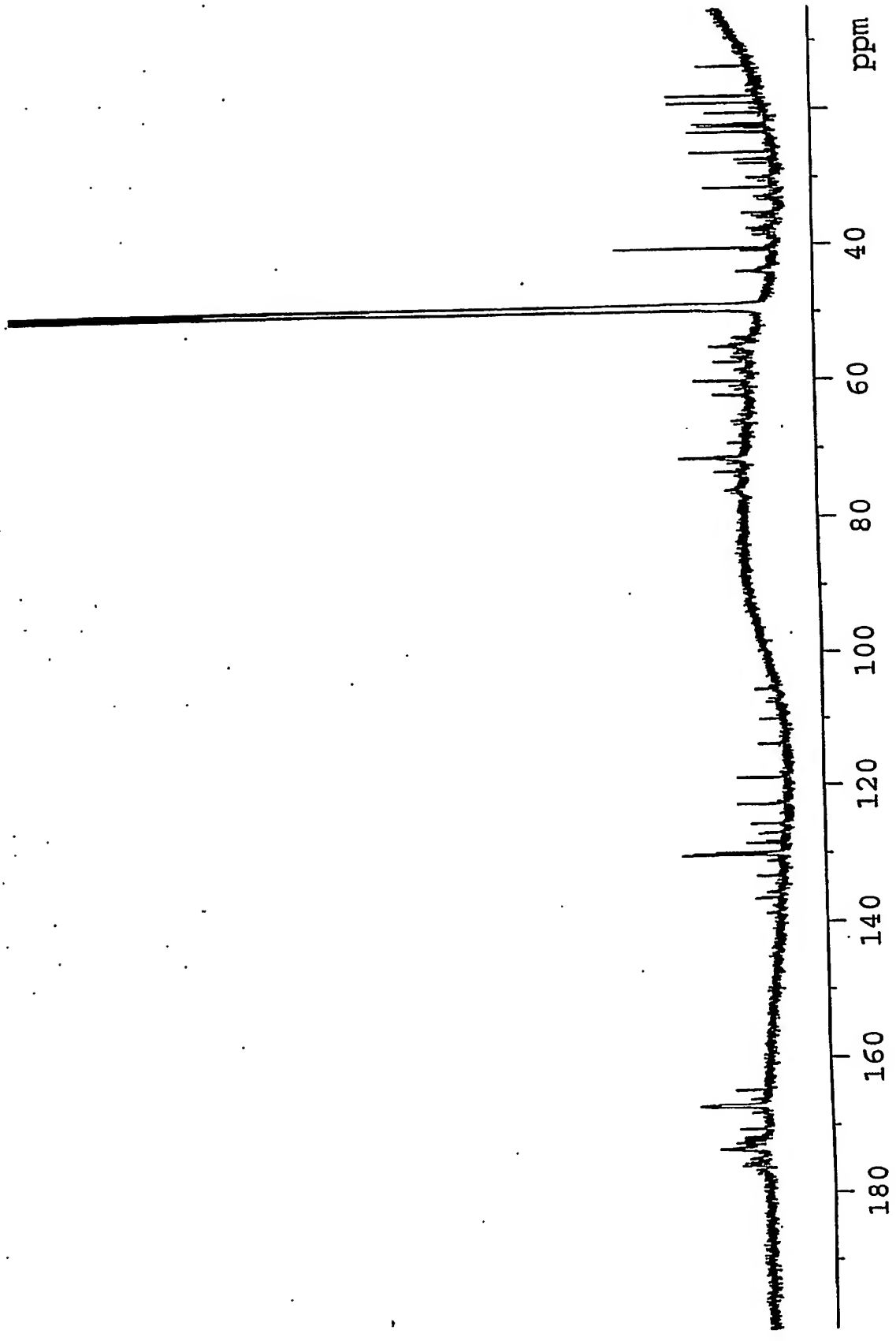
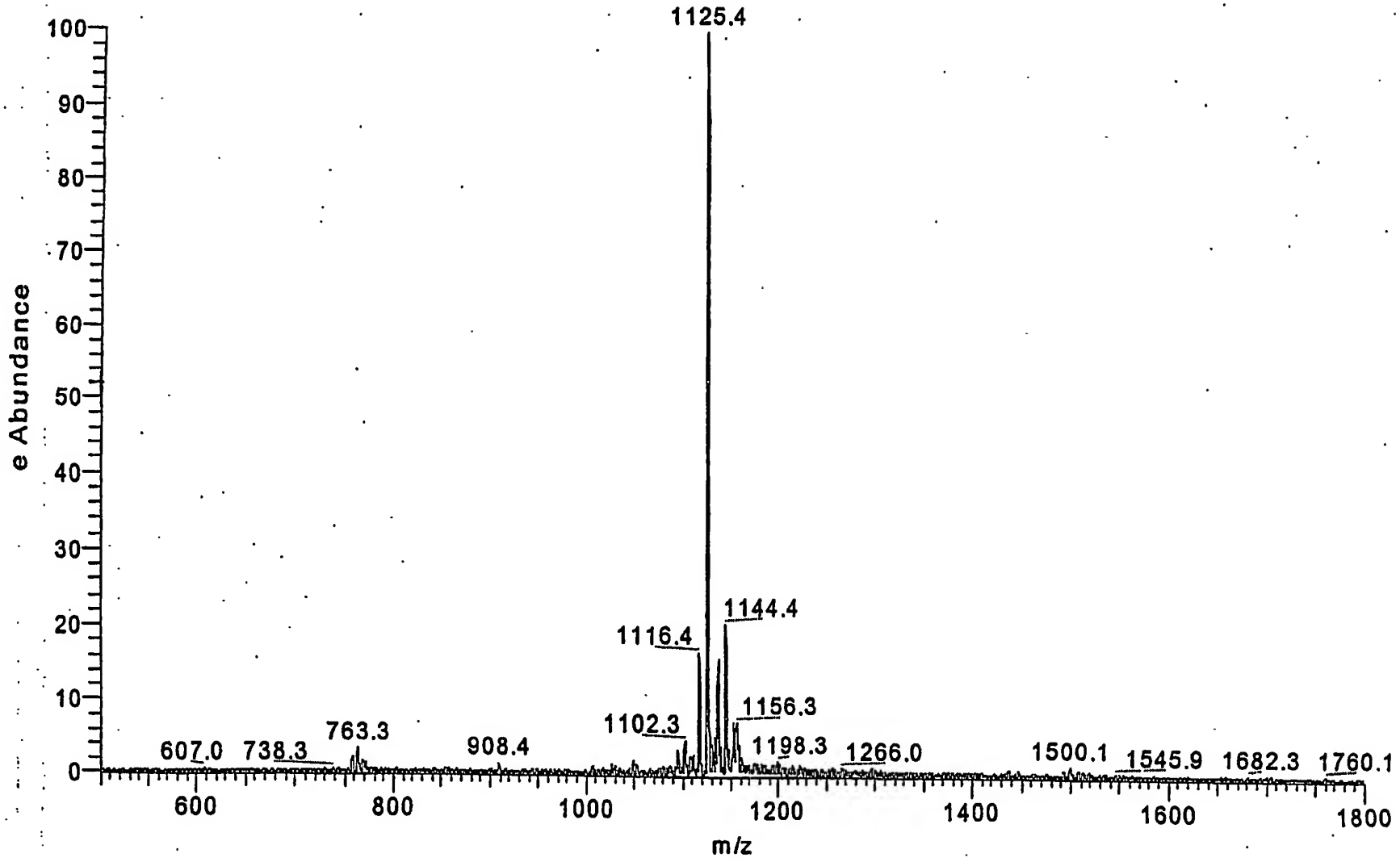


FIG. 5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100
90
80
70
60
50
40
30
20
10
0
e Abundance
1144.4
1116.4
1156.3
1102.3
763.3
607.0
738.3
908.4
1198.3
1266.0
1500.1
1545.9
1682.3
1760.1
600
800
1000
1200
1400
1600
1800
m/z

FIG. 6A

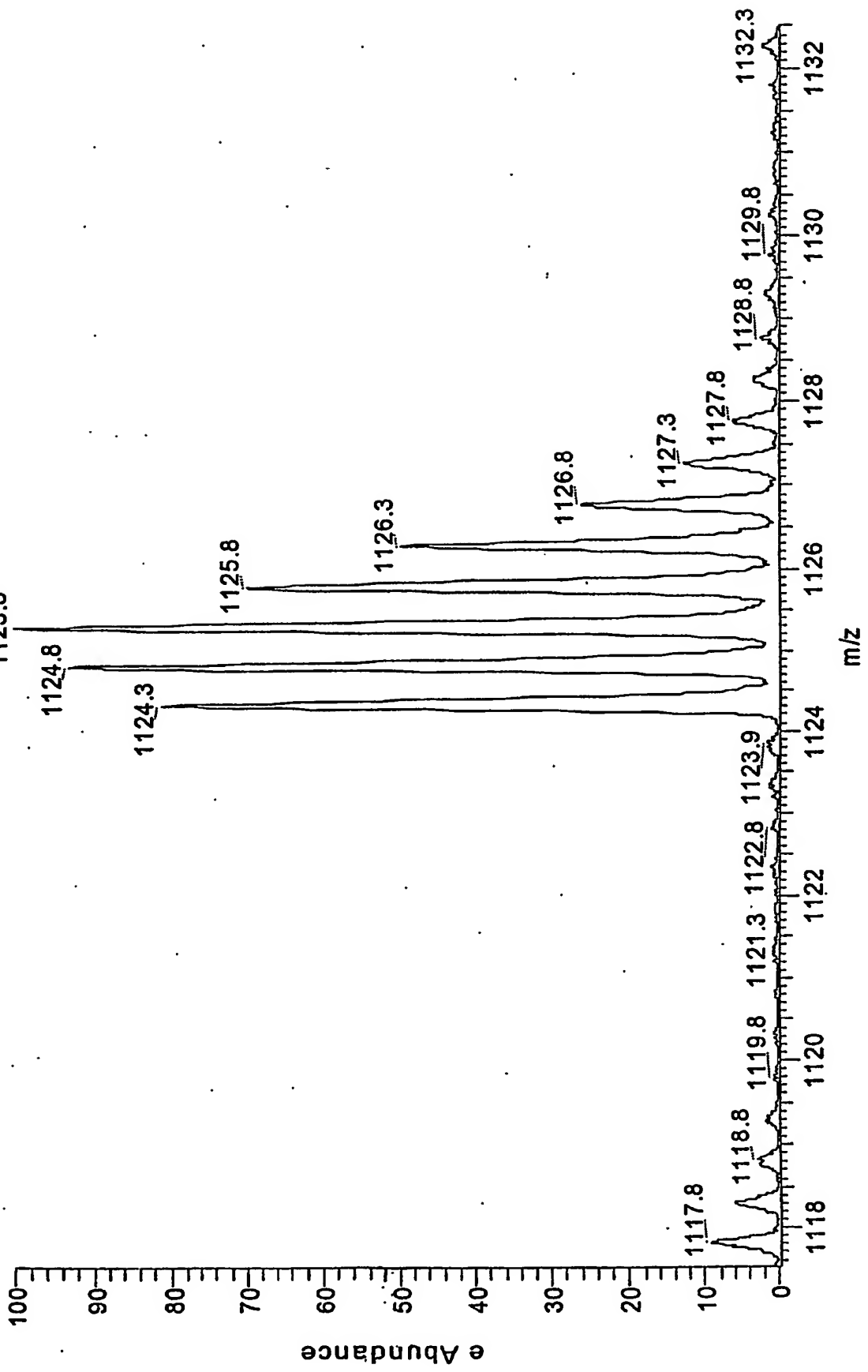
e Abundance
100
90
80
70
60
50
40
30
20
10
0
1118
1120
1122
1124
1126
1128
1130
1132
m/z
1117.8
1118.8
1119.8
1121.3
1122.8
1123.9
1124.3
1124.8
1125.3
1125.8
1126.3
1126.8
1127.3
1127.8
1128.8
1129.8
1132.3

FIG. 6B

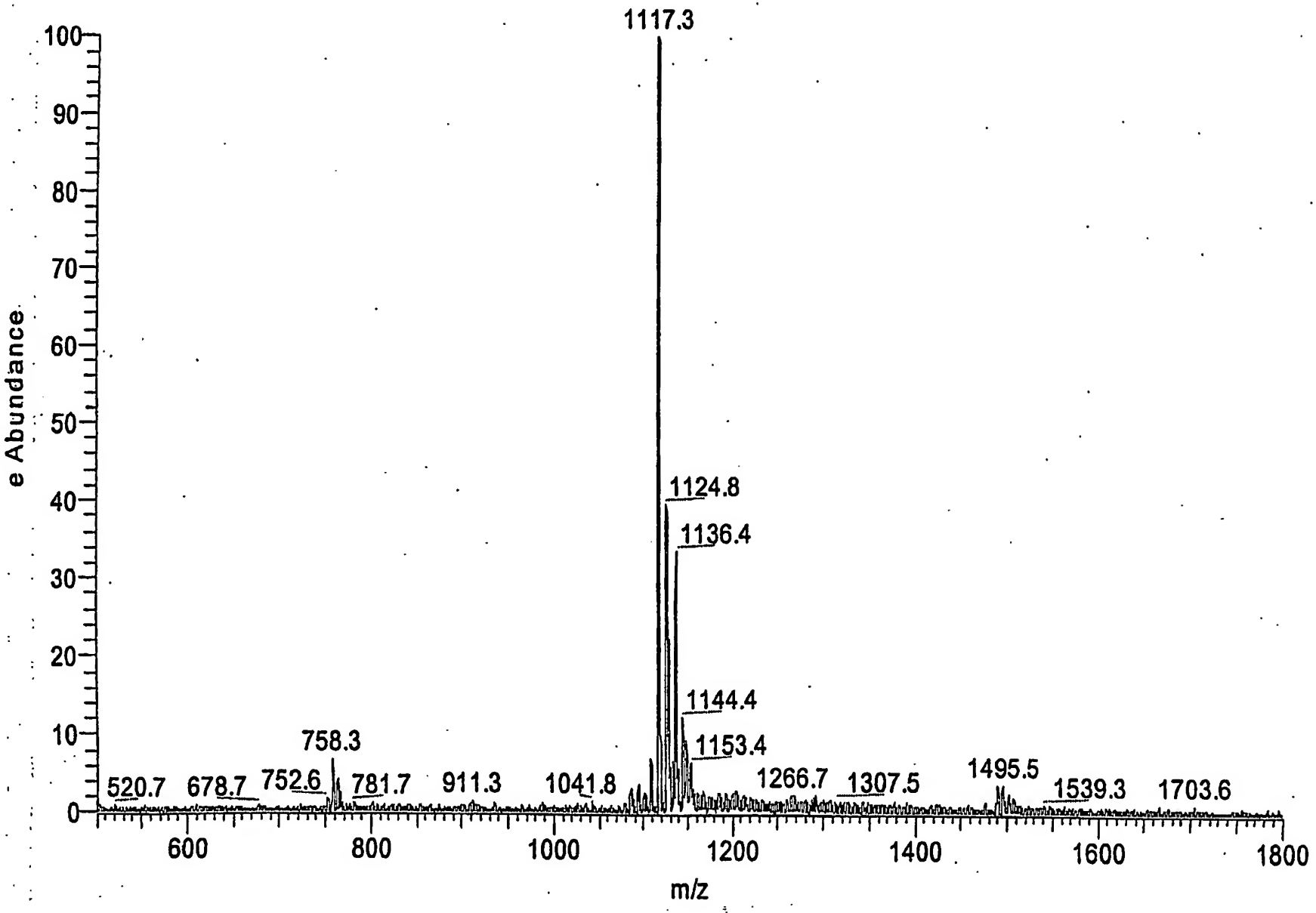
1117.3
100
90
80
70
60
50
40
30
20
10
0
e Abundance
1124.8
1136.4
1144.4
1153.4
758.3
520.7
678.7
752.6
781.7
911.3
1041.8
1266.7
1307.5
1495.5
1539.3
1703.6
600
800
1000
1200
1400
1600
1800
m/z

FIG. 7A

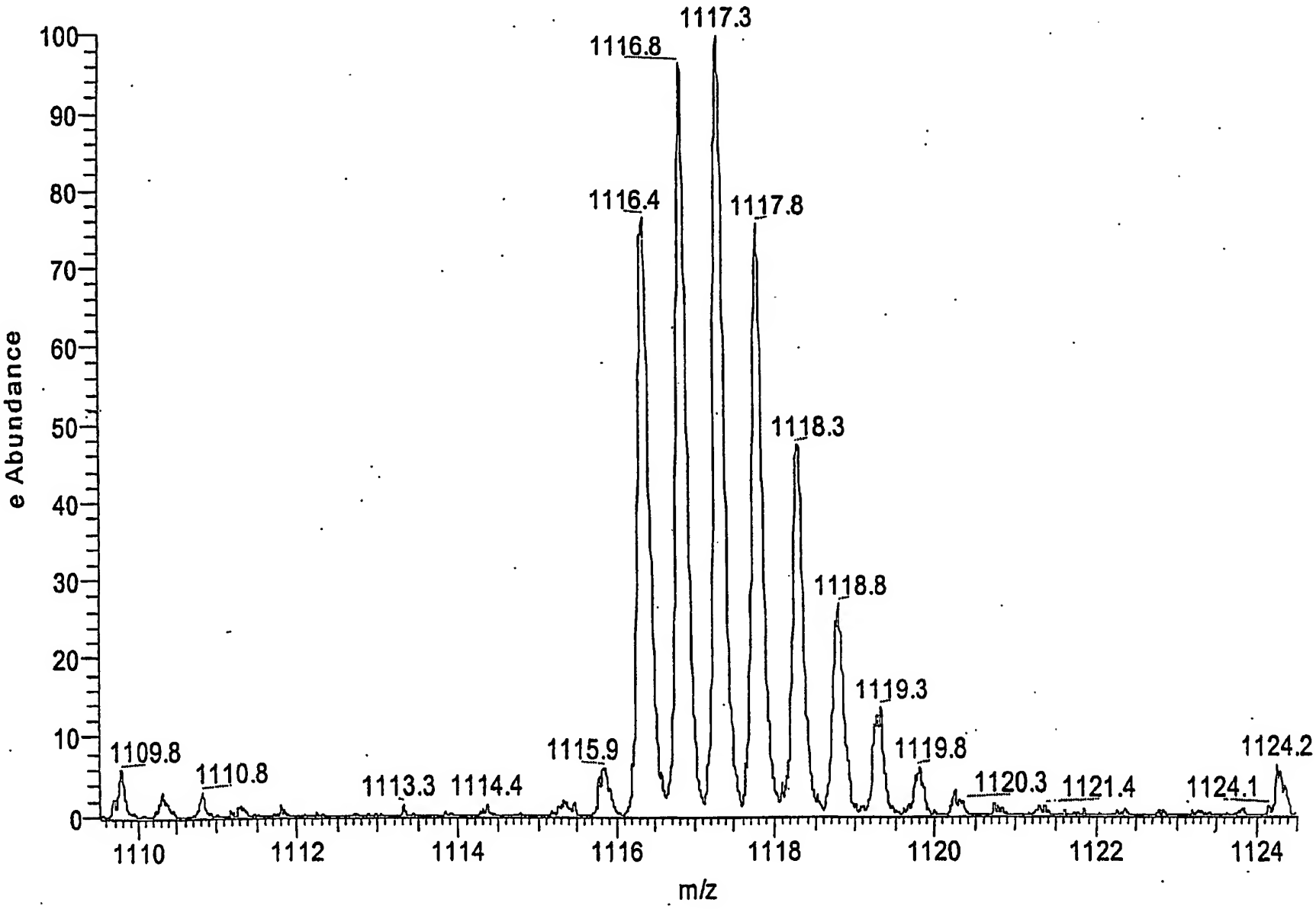
100
90
80
70
60
50
40
30
20
10
0
e Abundance
1117.3
1116.8
1116.4
1117.8
1118.3
1118.8
1119.3
1109.8
1110.8
1113.3
1114.4
1115.9
1119.8
1120.3
1121.4
1124.1
1124.2
1110
1112
1114
1116
1118
1120
1122
1124
m/z

FIG. 7B

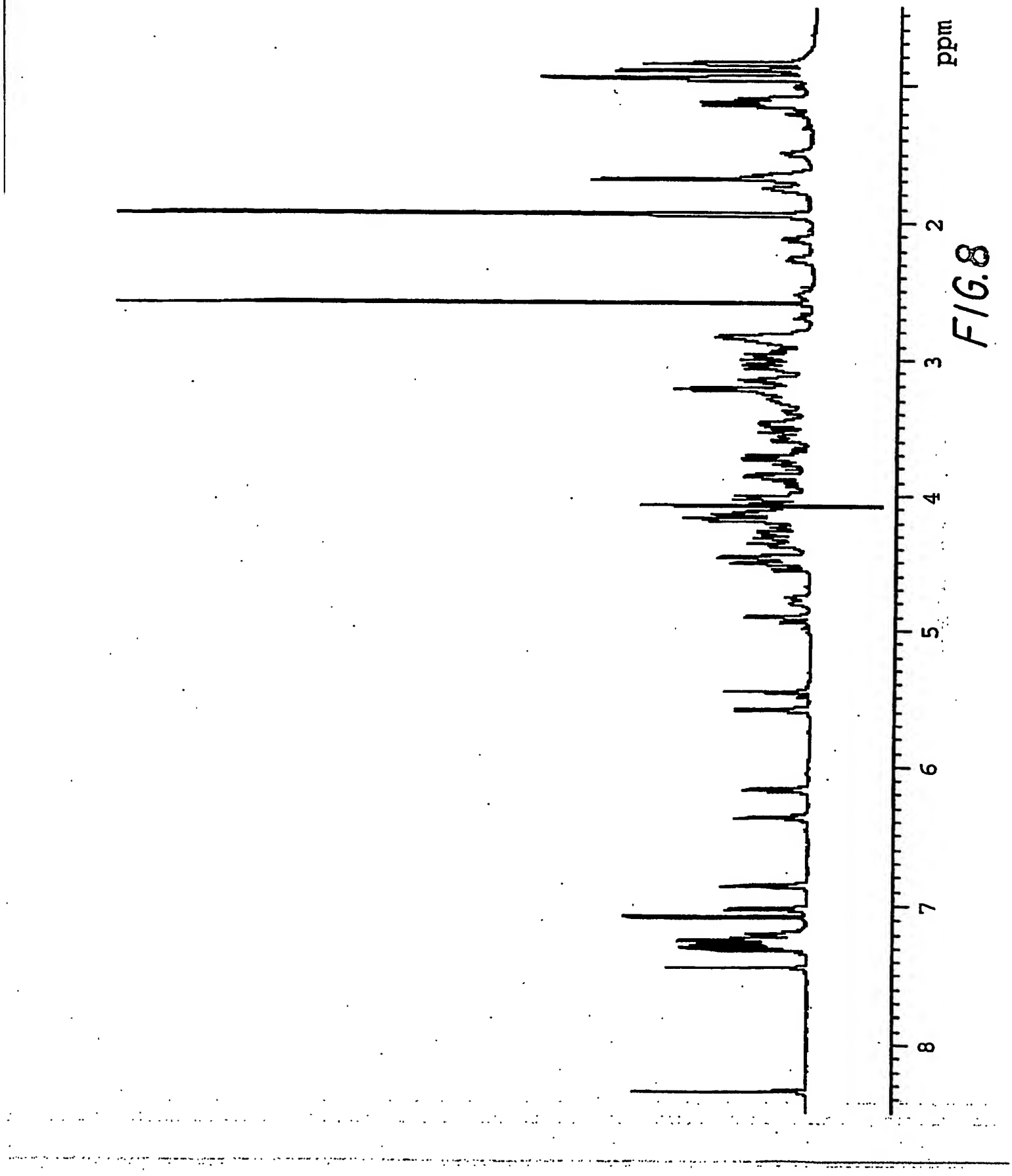

FIG.8

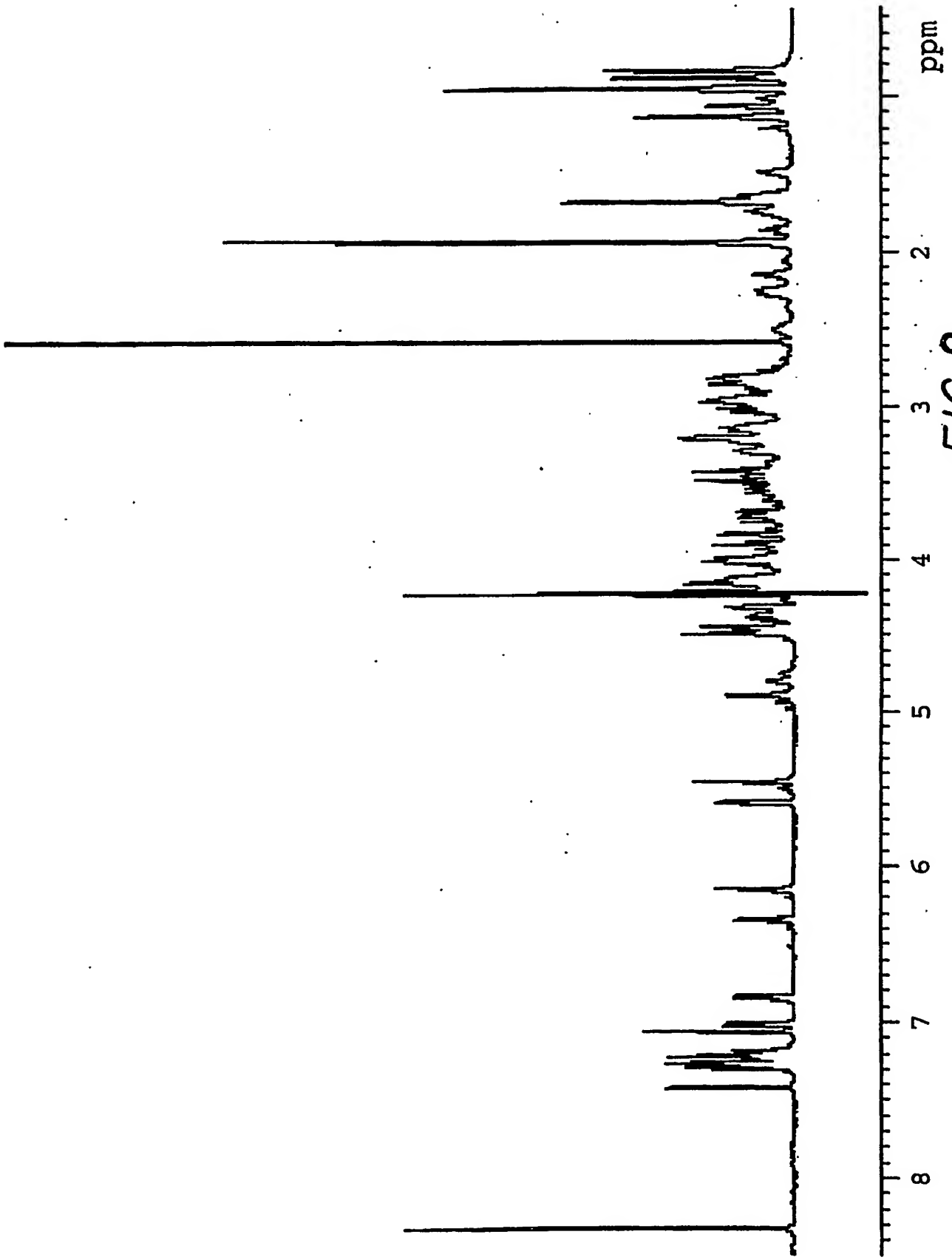

FIG. 9

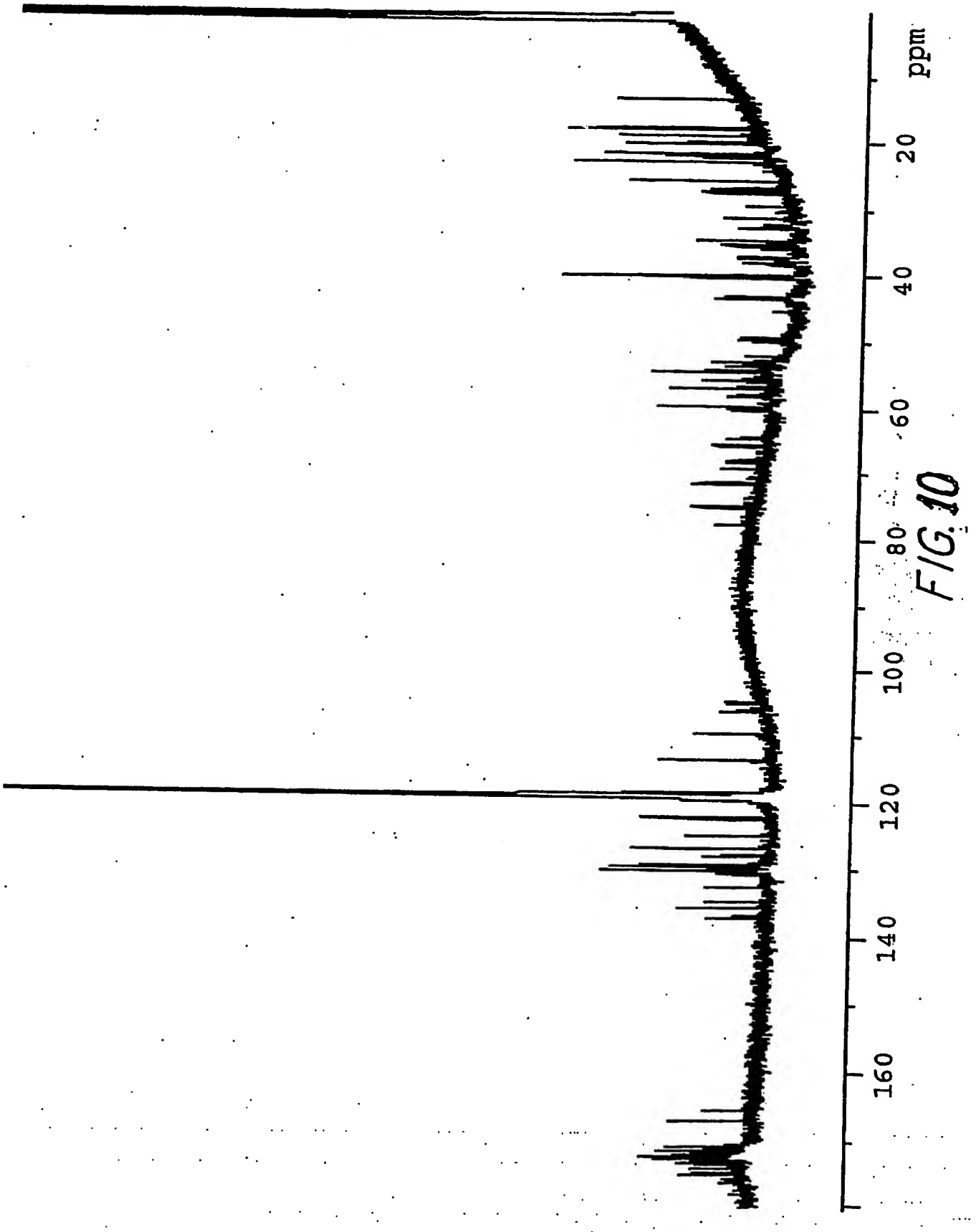

FIG. 10

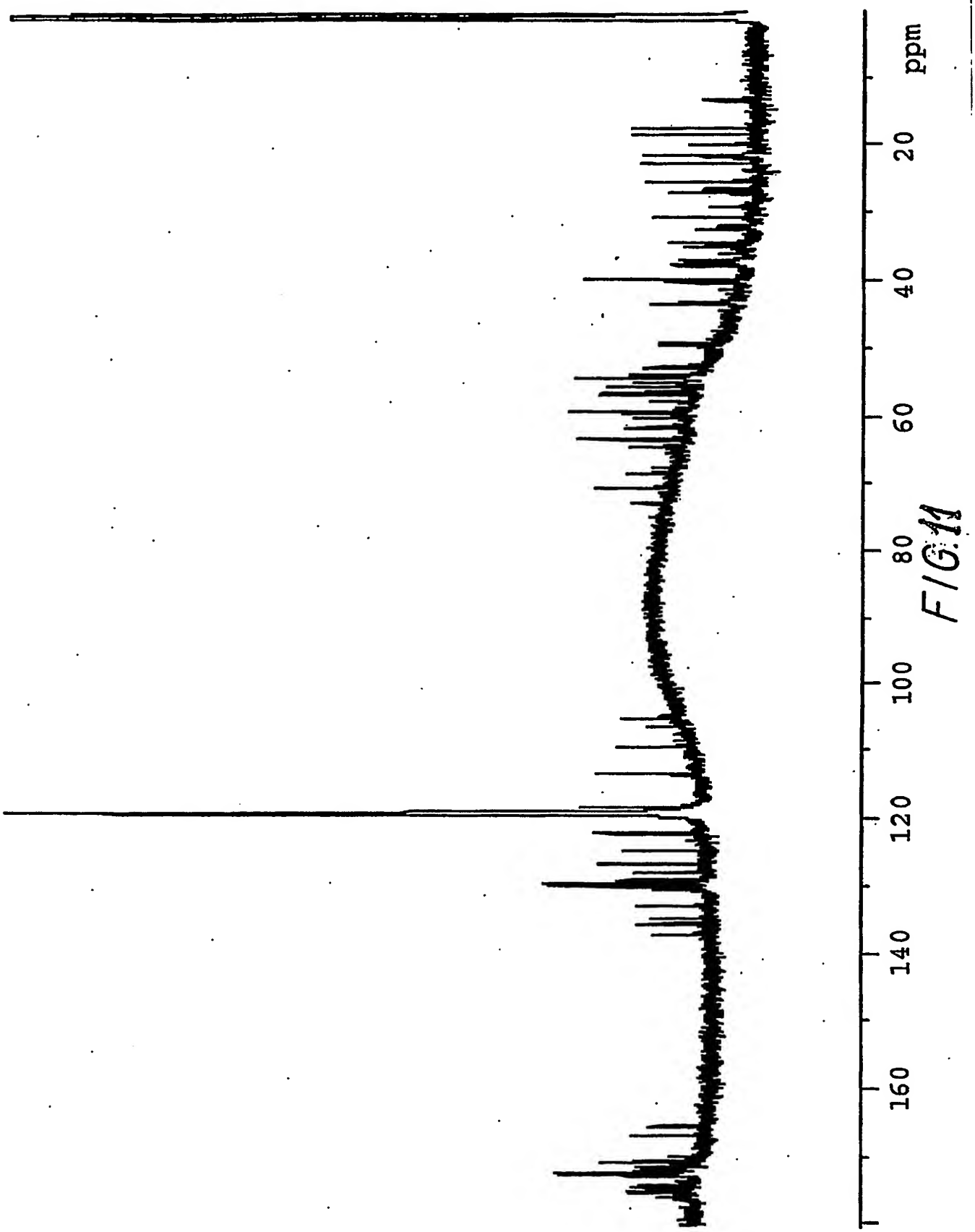

FIG.11